

Time for a New Look

THE inquiry to be carried out by the Council for Scientific Policy into the British government's machinery for scientific research (see *Nature*, last week) is overdue in the sense that it should have been undertaken several years ago. Necessity will not, however, ensure that all those affected by the inquiry will share in the general rejoicing that a thorough examination of civil science may now be carried out. One obvious difficulty, of course, is that if the inquiry means anything at all, its report is bound to argue for changes of significance in the present arrangements for spending money on research. At this stage, the fairest course is to identify the areas in which Professor Dainton's working party can most profitably function. Eventually, of course, there is bound to be a great deal of legitimate argument about the validity of the judgments which it makes.

The most obvious difficulty in the present arrangements is the increasingly awkward relationship between the University Grants Committee, the universities and the research councils. Theoretically at least, British universities obtain most of their research support from the UGC and the councils. Not so long ago, the received doctrine was that the research councils were public analogues of private foundations. They were expected to initiate lines of research and to hand these over to the universities when they were either established successes or known failures. On this basis, the universities and the UGC between them were supposed to make objective decisions about the balance of university research. Over the years, this doctrine of the transfer of responsibility for research from the councils to the universities has been allowed to lapse and for the best of reasons—it is now acknowledged even by the universities that outside sponsorship of research makes for flexibility and economies of scale. The difficulty is that the policy of the takeover has never been decently buried and, worse still, not sufficient attention has been paid to the problems there will be if the research councils (and principally the Science Research Council) are to be almost equal partners with the UGC in the financial support of the universities. How, in particular, will steps be taken to ensure that there is a fair balance of government and university influence in the making of policy for the research councils?

The role of the research councils themselves is much in need of definition. To be sure, the Science Research Council is increasingly clear-headed. Its interests are almost rigorously confined to the development of university research and of the facilities necessary to make university research effective. None of this has prevented the SRC from seeking to implement government policy, as with its attempts to encourage industrially valuable research at the universities, and all this is fair and above board. Naturally enough, the Science Research Council is least happy in its dealings with the polytechnics, but this is only a reflexion of the tentativeness with which the government as a whole regards these hasty creations of the previous government.

The clarity of the policy of the Science Research Council

is unhappily in sharp contrast with that of the other research councils, which tend to regard themselves as hybrids of academic research and sponsors and industrial entrepreneurs. The Agricultural Research Council is the worst offender—it tends to act the industrialist in its dealings with academics and the academic research sponsor in its dealings with farmers. The question which the Dainton working group should answer, where the research councils for agriculture, medicine and the natural environment are concerned, is whether there is a continuing case for institutions supposedly devoted to long-term practical goals yet institutionally separate from the organizations devoted to similar short-term objectives. In short, the working group will need to ask whether it remains sensible to adhere to the old Haldane doctrine on the organization of civil research. The argument must necessarily be particular, but there is at least a strong suspicion that Haldane has been hallowed for too long.

The most serious problem to be tackled in the field of civil research—the Dainton working party will not be encouraged to stray into defence research—concerns government policy for the support of industry. In retrospect, it must be acknowledged that the Labour government had the best intentions and almost the worst record imaginable. To the extent that the half-completed reorganization of the Atomic Energy Authority was an attempt to devolve responsibility from the government to laboratories such as that of Harwell or even to industrial consortia such as that which has assumed responsibility for the development of fast reactors in nuclear energy, the then government was of course anticipating the kinds of solutions likely to be most appealing to a Conservative, not a Labour, government. In practice, however, the devolution may have gone too far in some respects and not far enough in others. Specifically, there is now a serious danger that public involvement in fast reactor development is inadequate, but it would also have been better if the previous government had grasped some part of the nettle of the redundant laboratories.

Elsewhere, the Labour government should have practised more of what it was fond of teaching about the need to encourage technical innovation. Telecommunications is by now a quite familiar problem. It is increasingly evident that the new Post Office Corporation will for decades be too preoccupied with day to day problems to support the long-term research necessary to make full use of such things as bulk microwave transmission, satellite broadcasting and cable television systems. In the old days of the Haldane doctrine, no doubt, the solution would have been to set up a Telecommunications Research Council but that prospect would now be an entirely dead duck. There is much more to be said for an industrially oriented but substantially autonomous research authority constructed around the existing government laboratories with an interest in the field, especially the Royal Radar Establishment at Malvern. And the same pattern is probably applicable in other fields—aircraft for example. With luck, the government



could hope to share some of the costs with industry, although that should not be a prerequisite for the setting up of organizations like this. Ironically, of course, the arguments in favour of such an organization for telecommunications lack analogues where agriculture or even medicine is concerned. There, it will be surprising if the Dainton working group does not seriously consider

a separation of responsibilities for academic research (which could conveniently be transferred to the Science Research Council) from the more secular responsibilities of the two organizations (which might with advantage be amalgamated with those of the scientific establishments now separately maintained by the Ministries of Health and of Agriculture).

Are Institutions capable of Change?

DR DONALD SCHON has been one of the more successful of the BBC's public monuments to its first director-general. In his series of Reith Lectures, Dr Schon has talked about the interaction between the forces tending to change society, especially technology, and the institutions which have in the past given society its sense of permanence, some say smugness. It is, of course, almost inevitable that Reith lecturers should find themselves tempted into discussions of this familiar problem—on such a conspicuous public platform, most other subjects must seem outrageously inadequate.

The undertone to the Reith Lectures has been sombre (which is not the same as gloomy)—Dr Schon considers that there has been such a transformation of the pace of change in recent years that what he calls the belief in the “stable state” of society has disappeared. People no longer expect that institutions will do what has been traditionally expected of them. Survival entails that people should be prepared existentially to work things out as they go along.

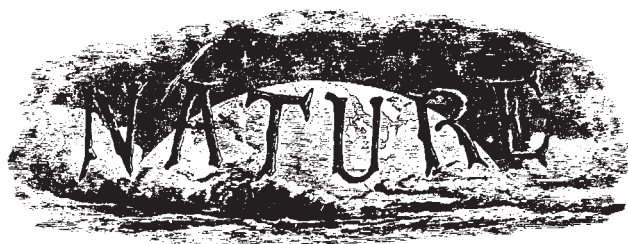
As in all questions like these, the concept of the stable state is necessarily subjective—Dr Schon admits as much. One of the necessary assumptions of civilized life is that existing institutions and current conventions can be supposed to be valuable until they are found by experience to be faulty. If, for example, countries such as Britain find themselves saddled with the institutions of parliamentary democracy rather than with those belonging to a more formal and more explicit constitution such as that of the United States, it is nevertheless worth persevering with the machinery which is to hand at least in the sense of supposing that difficulties which become apparent are to be accepted as difficulties in their own right and not as faults within the system. Thus, for example, if the British economy should be afflicted—as it is—by a severe bout of inflation, the sensible course is to seek some way of using the machinery that exists to put the economy right rather than to ask whether a radical reform of the machinery of government would be the best solution. The proper course is at least to see whether the existing institutions are sufficiently tough and flexible to deal with the problems they confront.

Does it follow that institutions should never change? Is it never right that institutions should be discarded? Nothing in the view that existing institutions are to a first approximation adequate can justify inaction or blind conservatism. For one thing, the mere process of using existing institutions to solve new problems brings change. This, for example, is the spirit in which research councils in Britain have introduced schemes for encouraging academic research of potential value to industry. In some circumstances, the interaction between institutions and the events with which they deal is so fruitful that the institutions can keep alive for decades or even centuries.

Elsewhere, it may become painfully apparent that institutions once sufficient for their purposes have become outmoded and even useless. One of the practical problems in social reform is to distinguish between the institutions that are worth preserving and those which might be got rid of. The present concern about the British Association for the Advancement of Science is, in its small way, a poignant example of the dilemma of whether to keep alive or to discard and start again.

One of the most serious weaknesses in Dr Schon's argument is the unavoidable difficulty of demonstrating that present circumstances are exceptional. Dr Schon says that “it is apparent” that “the threats to the stable state now exceed our various strategy for defending it” but goes on to quote as evidence the way in which the United States has allegedly ceased to tolerate what Dr Schon describes as “the great imbalance between the product-based consumer society and the requirements of the public system”, the discontent with the position of minorities and the disenchantment of large numbers of young people with the social goals of recent decades. Dr Schon is no doubt right in asking for a greater awareness of the functions and the limitations of social institutions, but there is nothing in his argument to demonstrate that this need has recently and suddenly become apparent.

100 Years Ago



THE frost which has now lasted for a fortnight is the most severe that has been known in England since the memorable one of Christmas, 1860, that is, for exactly ten years. The lowest temperature at Blackheath was 15.3° F. on the night of the 24th December; but in the eastern counties the cold was more intense, being 8° at Hull, and nearly as low at Norwich, Nottingham, and Leicester. The highest minimum recorded by Mr. Glaisher in the *Gardener's Chronicle*, at any English station is 19.0°, at Leeds. In Scotland the minimum varied between 5.0° at Perth, and 19.2° at Aberdeen. The average was slightly higher in Scotland than in England. For the first fourteen days of the frost, the temperature scarcely rose above the freezing-point night or day, a very unusual circumstance in this country.

From Nature, p. 193, January 5, 1871.

OLD WORLD

CERN ACCELERATOR

One More Delay

by our Special Correspondent

Geneva, December 22

A FINAL decision to build the CERN 300 GeV accelerator has been delayed yet again. Contrary to expectation, the CERN Council was unable to give the go-ahead when it met in Geneva on December 22, and the meeting was adjourned until February 19. Although the British government's decision to participate in the project removed one of the chief stumbling blocks, the strings attached to this decision together with some dithering by the governments of five of the smaller member countries of CERN made it impossible for the Council to come to a firm decision.

All the member countries who contribute most to CERN—West Germany, Britain, France and Italy—have already announced their willingness to participate, and Belgium, Switzerland and Austria have also declared their support. Only 13 per cent of the cost of the accelerator, estimated at about £100 million, is still to be accounted for, because delegations from Sweden, the Netherlands, Denmark, Norway and Greece all said that their governments require more time in which to make a decision. The chief reason for postponing the decision in Geneva last week was the condition laid down by the British government that it could agree to back the accelerator only if all the other member states of CERN did likewise. The German delegation to the meeting expressed some irritation that the decision was to be delayed yet again. It clearly felt that the British government was trying to dictate the progress of the new project, and the German delegation also pointed out that three other states with large contributions had only demanded that a substantial majority of the finance should be accounted for before formal assent should be given.

The postponement of the decision until February will not lead to any delay in starting the construction work on the accelerator. Several loose ends remain to be cleared up in the preparatory study, carried out by Dr John Adams, the director of the project, and there is still no clear plan for saving the (SFr) 200 million in the existing CERN programme required to finance the new accelerator. But if the British government does not soften its attitude at the February meeting, Sir Brian Flowers, chairman of the British Science Research Council, may be placed in the unenviable position of watching helplessly as the project is paralysed by the abstention of one of the smaller member countries.

MERCURY POLLUTION

Incomplete Knowledge

THE Minister of Agriculture's decision to allow tuna fish contaminated with methyl mercury to be sold in Britain is, on the evidence available, a sensible one. The level of contamination is well within the safety limit adopted by many other countries, and the amount of tuna consumed in Britain is comparatively small. But the whole affair has emphasized how arbitrary are the safety limits, and how incomplete is the knowledge of the effects of ingestion of methyl mercury on the nervous system. The Laboratory of the Government Chemist found that the mercury content of tinned tuna fish sold in Britain is between 0.1 and 0.8 mg/kg.

Methyl mercury compounds are more toxic than the metal itself because they are soluble in fat, and can therefore penetrate cell membranes and enter the brain. One of the key questions is how long these compounds remain in the brain before they are removed, because the longer they are stored the greater are the risks of poisoning from small quantities ingested over a long period. Unfortunately, however, the residence time of methyl mercury in the brain cannot yet be accurately quantified, and this uncertainty casts some doubts on the validity of designated safety limits. Methyl mercury is thought to damage brain cells, thereby impairing the coordination of muscle control—the first clinical signs being numbness in the extremities, lips and tongue. The present safety limits are based on the number of brain cells likely to be damaged before any deleterious effects are noticed.

The chief sources of mercury contamination of the environment are pulp and paper factories which use phenyl mercuric acetate and, to a lesser extent, chlorine factories using mercury electrodes. Research carried out in Sweden has shown that bacteria in bottom sediments convert inorganic mercury to organic mono- and dimethyl (CH_3Hg^+ and CH_3HgCH_3) derivatives. The process seems to be an anaerobic, non-enzymatic fermentation, involving vitamin B_{12} . The bacterium *Methanobacterium omelianskii* ferments carbon dioxide and produces methane, a key step being production of methylcobalamin—a complex between methyl, cobalt and B_{12} . Methyl mercury is likely to be a by-product of such a fermentation.

The Pharmacology Subcommittee of the Committee on Medical Aspects of Food Policy, which advised the government that the level of mercury found in tuna fish is not sufficiently great to ban the sale of the fish in Britain, recommended also that the Ministry of Agriculture should initiate an extensive monitoring of all possible sources of methyl mercury in food. The committee also pointed out that the problem of mercury con-

tamination has been in existence for a long time and there has not been sudden exposure to a new risk.

COMPUTERS

Good Year for ICL

International Computers (Holdings) Ltd last year increased its turnover by 13 per cent, compared with the turnover during the previous year. Profits also increased dramatically—by 33 per cent, to £4.53 million after tax. This announcement, made last week by Sir John Wall, chairman of ICH, bodes well for the company's ability to meet strong competition from computer manufacturers in the United States and Europe, and this optimism is further strengthened by the announcement that 40 per cent of the company's turnover was achieved in foreign markets. ICH was formed in 1968 as a holding company for International Computers Ltd (ICL)—an amalgamation of International Computers and Tabulators and the computer interests of English Electric and Plessey, with the government holding some of the shares.

The company spent £16 million on research and development last year and Sir John Wall predicted that this will soon reach the £20 million a year mark. And for the first time, the company spent more on research on software than on hardware, reflecting the trend established during the past few years (see *Nature*, 228, 201; 1970). ICH has also spent most of the £13.5 million grant awarded by the Ministry of Technology in 1968 for research and development.

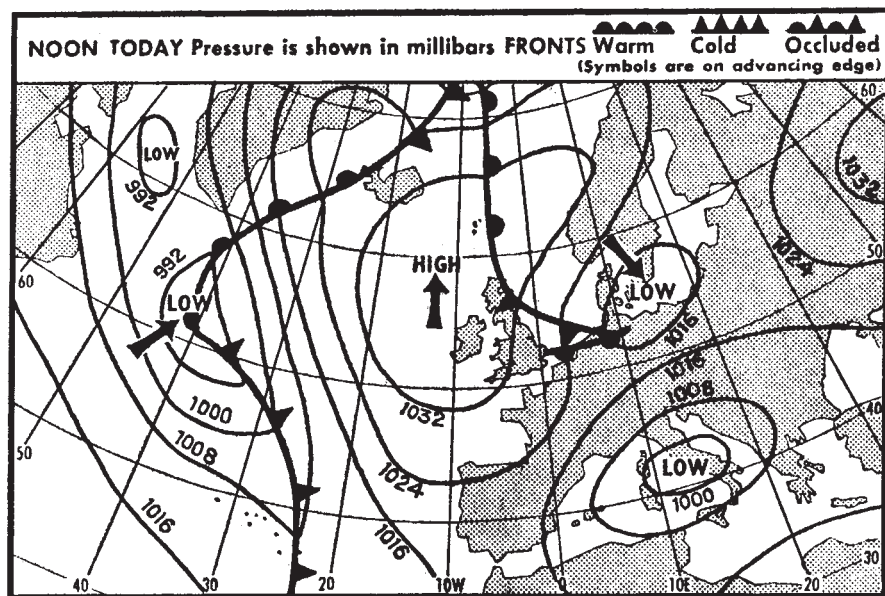
During the past year ICL has been looking towards closer collaboration with foreign manufacturers, and together with Control Data Corporation of the United States and Compagnie Internationale pour l'Informatique in France, ICL has set up a joint study company in Brussels to explore ways of collaborating. The first step will be to ensure that the software and hardware products of each company will be compatible, to facilitate any future joint marketing operation between the three companies.

As far as future ICL computers are concerned, Sir John Wall declined to give a date for launching its new series of computers. Although ICL does not intend to manufacture the type of giant computers made by Control Data Corporation, Sir John said that it will go ahead with development of the Project 52 large computer.

METEOROLOGY

Unusual Patterns

THE sudden arrival of snow in England, promptly on cue for Christmas, was less of a surprise to meteorologists than its disappearance earlier this week. The weather conditions in England were



Meteorological chart for noon, December 23. © Times Newspapers Ltd.

part of an overall pattern which produced snow in Southern Spain, where in some places it was the first December snow for over a decade, while the Scottish ski resorts found what little snow they had melting in unusually warm sunshine. But although this bizarre situation does not often arise, it is not unusual for winter conditions to be more extreme in south east England than in the north of Scotland.

Because of the widening of the North Sea with higher latitude, Scotland has a climate more typically maritime, and hence less subject to sudden changes in temperature, than that of southern England. The situation which produced the Christmas snow was caused by a large anticyclone which moved from the Atlantic over Scotland. Cold air curling round this high pressure area travelled over Scandinavia and northern Russia before moving towards England over the North Sea, appearing as an easterly or a north easterly wind. The airstream arrived in England still cold enough for precipitation to fall as snow, but it had picked up enough heat from the North Sea to be warmed above freezing point by the time it reached Scotland.

The Meteorological Office was, however, caught on the wrong foot by the sudden thaw early this week, which occurred just as the forecasters were predicting more snow. In this case, the dominant feature in the weather pattern was a low pressure area moving northwards across Europe. Unluckily for the forecasters, the actual windspeed turned out to be rather lower than they predicted and the cold air warmed sufficiently during its passage over the sea to thaw out all except the extreme south eastern tip of England.

Just before Christmas, the unusually warm autumn had left the North Sea a

full degree centigrade warmer than usual for the time of year. All of this surplus heat store was lost during the curious behaviour of the last ten days, so that the overall picture now looks more typical for Europe and the North Sea in winter, making future predictions somewhat easier.

NRDC

New Man at the Helm

THE National Research Development Corporation has at last appointed a new managing director. The post, which has been vacant since the resignation of Mr John Duckworth in July 1970, is to be filled by Dr Basil Bard, an industrial chemist, and chairman of Advanced Materials Engineering Ltd. The corporation has been advertising for a new managing director in the national newspapers during the past six months, and has had some 150 applicants. But, ironically, the appointment of Dr Bard has been made from the inside—he has been a member of NRDC's board of directors since 1956, and he is chief executive of the corporation's Department of Applied Science.

The delay in appointing a new managing director can be explained in part by uncertainty about the future for the NRDC. The green paper on government research, published a year ago by Mr Anthony Wedgwood Benn when he was Minister of Technology, suggested that the corporation should form part of the proposed British Research Development Corporation, but the change of government last June inevitably means that many of the proposals outlined in the green paper will not be put into effect. Until the government outlines its policies for

government research, the position of NRDC remains uncertain, but it seems that there will be little change in its structure in the near future. The corporation has shown a small profit on its revenue account during the past two years, thanks chiefly to sales of the antibiotic cephalosporin, and the future for Tracked Hovercraft Ltd, a subsidiary of NRDC, looks bright.

SOVIET UNION

New Science Centre

from our Soviet Correspondent

A FAR EASTERN Science Centre of the Academy of Sciences of the USSR is being formed from existing scientific institutions in the Soviet Far East. The centre, which is to specialize in research connected with the natural resources and economic needs of the area, will serve a region stretching from the permafrost of the Arctic tundra, down to the subtropical forests of Sikhote-Alin' and from the Amur and Assuri rivers to the Pacific coast.

The centre's headquarters will be in Vladivostok, where institutes of geology, soil biology, marine biology, and biologically active substances have already been established. The centre will also incorporate the existing institutes in Khabarovsk, Magadan and on Sakhalin Island, and the Institute of Vulcanology in Petropavlovsk-Kamchatskii, which is engaged on field research on active volcanoes.

According to the articles of its foundation, this new centre will undertake "basic research in the natural and social sciences, the working out of scientific problems facilitating the more rapid development of the economy and productive forces of the Far East, the training of qualified scientific personnel and the coordination of scientific research undertaken by the institutes of the various ministries and departments situated in the Far East".

Writing in more practical terms, Academician A. P. Vinogradov, vice president of the Academy of Sciences of the USSR (*Pravda*, no. 352; 1970), emphasizes the importance to the programme of the centre of investigations of the bed of the Pacific, the study of the volcano belt and ore zones, including the investigation of the effect of the magma on tectonic and ore-producing processes. New surveys are to be undertaken, to find further ore seams and oil-gas deposits, and for this purpose an institute of geology and tectonics is being organized in Khabarovsk. It is also hoped to carry out a large-scale study of the geography and economics of the whole area. Institutes of physics and chemistry should also be established in the near future.

Birth of the Open University

by our Education Correspondent

THE Open University, after a long and complicated gestation period, will next week see the light of day. It remains to be seen whether the project will be still-born, or whether it will reach maturity, but it is nevertheless something of a triumph for its supporters that the plans have matured this far. Conceived by Mr Harold Wilson in 1963, and steered through its development by Miss Jennie Lee, the Open University is a bold and ambitious plan to make higher education available to a large number of people who have been denied the opportunity of going to a traditional university. Its potential impact on educational development can be judged in part by the fact that when the first broadcasts are transmitted on the radio network and television channels next week, nearly 25,000 students will be listening in. This one university will therefore account for nearly a third of the total number of students in first year courses at British universities.

But the road to the formal opening next week has not been an easy one, and perhaps the university's largest headache has come during the past few months. After the Conservative government gave the official go-ahead for the project last August, the university offered places to 25,000 of the 42,000 applicants. But by November it was clear that some 20 per cent of the successful candidates had already decided that they did not want to go ahead with their studies—and had not paid their initial £10 enrolment fee. Although the Open University maintains that such a large falling in interest among the students is not too alarming, there is no doubt that it is something of a setback. It also seems to have caught the university's planners by surprise because the reserve list was too small to fill all the available places and some re-selection had to be done.

But in many ways, the so-called drop-out rate is only one part of the university's worries, and it will even be something of a surprise if more than half the students who register stay the course. The studies will not be easy. To get a degree, students must build up a series of credits—six credits will be required for a BA degree, and eight for a degree with honours. Each credit will be awarded for successful completion of a one-year part time course, and it is expected that no more than two courses will be taken in each year. Tuition will consist of a system of correspondence courses, television and radio lectures, and tutorials

conducted on a regional basis. Students will also have to spend two weeks each year at a summer school. With these demands on a student's time, a strong commitment will be needed to qualify, and the 20 per cent drop-out rate which has been tentatively suggested for the university is very much an unrealistic estimation.

To the delight of the Open University's many sceptics, it is already clear that the original intention in the back of Mr Harold Wilson's mind will not be fulfilled. Mr Wilson clearly envisaged the university as a vehicle for enabling manual workers to have an opportunity of taking a degree, but applicants to the university turned out to be almost as overwhelmingly drawn from the middle classes as those to the traditional universities. In spite of preferences being given to candidates from the lower economic groups, the class bias among the students has not been ironed out, but the chances are that if the project is allowed to mature a larger proportion of the applicants to the Open University will come from manual workers.

One of the chief purposes of the Open University is to provide a second bite at the higher education cherry for those students who for one reason or another have missed out on their first opportunity. But it seems likely that this function may also be modified in the near future. The university is at present accepting applications from students aged at least 25: in other words, they will already be in employment, and the Open University will not be vying for candidates with the traditional universities. But the Department of Education and Science, casting around for ways to accommodate the demand for higher education during the next few years, has asked the Open University to look into the possibility of accepting students at the age of 18. Such a change in policy would have several repercussions—for one thing, the university would find itself attracting many more students who had been rejected by traditional universities, and the other universities would themselves be rather wary of the possibility that the Open University may take away some of their potential candidates. But, paradoxically, taking students at the age of 18 would be a good trump card for the university to play to ensure that it is allowed to carry on.

The Conservative party, which was in Opposition when the plans for the Open University were first aired, has always been sceptical of its chances of success,

but when the Conservative government took office in June this year, plans for the institution were already too far advanced to be called off. It now seems that if the university is to be scrapped, the first move will be to cut down on its grant and therefore on the number of students that it will take. The present plans are for a total student population of between 36,000 and 42,000 for 1972 and 1973, but no sanction has yet been given for estimates beyond that date. If, however, the Department of Education and Science sees room in the Open University for relieving the pressures on traditional universities, the future of the university will be more secure.

What, in any case, are the immediate plans of the Open University? Students starting next week will be taking one or two foundation courses in mathematics, science, humanities and understanding society, and next January a foundation course in technology will also be introduced. The six credits needed for a BA degree must include two from successful completion of foundation courses. Most of the broadcasts and correspondence material for this year's foundation courses have already been prepared, and the staff of the university is planning the second year courses. It is intended at present that a student's choice of foundation courses should not limit his choice of second year courses too much. But to take second year courses in science and mathematics, students must have completed foundation courses in those subjects.

As far as postgraduate studies are concerned, there are no plans to offer places for part time students until at least 1973—at present, there are only a few places available for full-time research students at the Open University's premises at Milton Keynes. But the university intends to introduce updating and post-experience courses to help movement between occupations.

It will not be possible, however, to assess the real contribution of the Open University to higher education in Britain until its first graduates have been produced and have started looking for new jobs. Only then will it be possible to tell whether degrees from the Open University are considered by employers to be on a par with those from traditional universities. Unless this is the case, Open University students will have good cause to complain that their investment in terms of both time and money has been a bad one.

NEW WORLD

Darwin shares with Eve and Adam

by our Washington Correspondent

THE conflict between the scientific and biblical version of man's origin is a topic of perennial interest, but one which, as far as concerns the content of biology textbooks, had ceased in most countries to be a public issue within a few decades of Darwin's death. Not so in the United States, where those who favour the most literal interpretation of the myths of *Genesis* have been able repeatedly to put to rout the feeble forces of reason. Admittedly, the anti-evolution laws in many southern states have long been a dead letter, but that does not mean that fundamentalism in America is a paper tiger. Just as these harmless legal curios are being swept off the statute books of Mississippi and Tennessee, the state of California has embarked on a course which could give fundamentalist ideas their first foothold in biology textbooks.

The fundamentalists' most famous victory, though Pyrrhic in retrospect, was the "monkey trial" at Dayton, Tennessee. The echoes of the great debate between Bishop Wilberforce and T. E. Huxley had long continued to reverberate in the states of the Bible Belt and they swelled to a crescendo in 1925 through the efforts of one John Washington Butler, the clerk of the Round Lick Association of Primitive Baptists. He succeeded in adding to the statutes of Tennessee a law which forbade the teaching in schools of any theory that seeks to deny "the story of the Divine Creation of man as taught in the Bible, and to teach instead that man has descended from a lower order of animals". The testing of the law made for a trial that astounded the world; the jury was less impressed, taking only eight minutes to reach the verdict of guilty and impose a \$100 fine on John T. Scopes, the young biology teacher who had agreed to stand as defendant.

The views of the Tennessee primitive baptists and their brethren in other states had small effect and less than half a century's permanence in law. The Tennessee law was repealed in 1967, and in 1968 the Supreme Court ruled that an Arkansas anti-evolution law was unconstitutional since it violated the First and Fourteenth Amendments. In December 1970 the bastion of Mississippi surrendered to a Mrs Arthur G. Smith when an appeal court overturned the state anti-evolution law, the last of its kind in the United States. With the death of John Scopes in October last year, who after the Dayton trial led as blameless and uncon-

troversial life as he had before it, the era of legal intervention in the teaching of evolution should have come to a close, a historical cul-de-sac no more capable of resurrection than Trofim Lysenko or the inquisitors of Galileo.

But though the fundamentalist cause may have died away in the Bible Belt, it has staged a remarkable second coming in California. There the State Board of Education has propounded, with rather less than the wisdom of Solomon, a doctrine which would apparently have biology textbooks give equal weight in discussing how life began to the opinions of the authors of *Genesis* and to those of modern science. The doctrine is expressed in the form of a text inserted by the board into a guidebook for teachers known as the Science Framework for California Public Schools. So far, the doctrine has had little impact on school teaching, but where its effect could be more seriously felt is in the selection of new textbooks, which must be submitted to the board on June 1. The significance of the situation is that California schools account for some 10 per cent of the textbooks used in the United States, and unless publishers are prepared to forgo this market, much of the nation could find its students reading biology textbooks altered to suit the decrees of the twelve members of the California State Board of Education.

The text of the doctrine is as follows:

All scientific evidence to date concerning the origin of life implies at least a dualism or the necessity to use several theories to fully explain the relationships between established data points. This dualism is not unique to this field of study, but is also appropriate in other scientific disciplines such as the physics of light.

While the Bible and other philosophic treatises also mention creation, science has independently postulated the various theories of creation. Therefore, creation in scientific terms is not a religious or philosophic belief. Also note that creation and evolutionary theories are not necessarily mutual exclusives. Some of the scientific data (e.g., the regular absence of transitional forms) may be best explained by a creation theory while other data (e.g., transmutation of species) substantiate a process of evolution.

Although the doctrine is open to various interpretations, it seems to be saying that the biblical and scientific explanations of the origin of life are

alternatives of equal validity, just as the wave and particle theories of light are different but equally valid explanations of a single phenomenon. According to Mr Frank Mann, the science consultant to the board, there is no intention of "putting the Bible into science textbooks". Although nobody seems sure of just what the board does have in mind, Mr Mann believes the doctrine to be saying simply that the scientific explanations should not be presented to the exclusion of religious ones in biology textbooks: "We need to qualify our statements about evolution to indicate that this is not necessarily the only or final answer", is the science consultant's interpretation.

What made the Board of Education adopt this position? The pressure to which California yielded came almost entirely from a 1,500 member organization known as the Creation Research Society. The society, which has no income other than its \$7 annual membership fee, was founded in 1963 by Dr Walter E. Lammerts, a prize rose breeder and former professor of horticulture at the University of California, Los Angeles. The 300 voting members are all scientists with MSc or PhD degrees, and all subscribe to a fundamentalist view of the origin of life. The charter of the society commits members to "full belief in the Biblical record of creation and early history, and thus to a concept of dynamic special creation (as opposed to evolution), both of the universe and the Earth with its complexity of living forms".

The purpose of the society is to publish its own journal, the *Creation Research Society Quarterly*, since it was discovered that scientific journals refused to publish papers expounding the society's views. The editor of the quarterly, Dr George Howe, a botanist who is chairman of the science department at the Los Angeles Baptist College, says the journal goes to the 1,300 members and to a few libraries, with a total circulation of less than 2,000. The society's only interest is in publications and it is not a pressure group, although individual members may lobby for the society's views.

It was three non-voting members of the Creation Research Society who induced the California Board of Education to recant on its single-minded allegiance to science. The three women members, Elwyn Angle, Nell J. Seagraves and Jean E. Sumrall, appeared at a public hearing in 1969 held to approve the new

Science Framework prepared by the State Advisory Committee on Science Education. Their contention that the framework gave unfair precedence to evolution to the exclusion of religious explanations persuaded the chairman of the Board of Education, Dr Max Rafferty, and two of his colleagues, to send the framework back for redrafting. At a second public hearing held on November 13, 1969, the science education subcommittee came back with substantially the same version of its framework to which the Creation Research Society members again raised objection. Over the protests of its science education subcommittee, the Board of Education then substituted into

the framework the text quoted above which, as it happened, was written not by the Creation Research Society but by a member of the American Scientific Affiliation, a group devoted to studying the interaction between science and the Christian faith.

Dr Rafferty was replaced in last year's elections (the chairmanship of the Board of Education being an elective post) and his successor may lead the board away from the precarious ground it has entered. But failing a change in the board's position, publishers with an eye on the Californian market will soon have to decide how to meet the requirement for a dualistic presentation of the origin

of life. The only publisher whose textbook is already prepared according to these specifications is the Creation Research Society; called *Biology—A Search for Order in Complexity*, the textbook "gives the evidence from both sides".

If the textbook is adopted by the Board of Education, California biology classes will be learning their science from people who believe that *Genesis* is a "factual presentation of simple historical truths", that all living things, including man, were made by the direct creative acts of God during the Creation Week, and that the Noachian flood was a historical event.

Cleaning Up for Sweden, 1972

from our New York Correspondent

AFTER TWO and a half years of work, the slowly grinding cogs of international negotiations are about to produce an agenda for the United Nations' first Conference on the Human Environment due to be held in Sweden in the summer of 1972. The conference was first proposed by the Swedish government in May 1968, and since then the planning has been gradually working through committees of the Economic and Social Council and the General Assembly.

Since Mr Maurice Strong was appointed in September as the United Nations' Under-Secretary for Environmental Affairs and secretary-general of the conference, he has begun to build a staff and to arrange the second preliminary meeting in February at Geneva. Mr Strong comes to the United Nations with a reputation as something of a dynamo in the Canadian government. A self-made millionaire, he left industry in 1966 to become president of the Canadian International Development Agency.

Mr Strong's previous post should prove especially useful since many developing nations have already attacked the conference as a plot by the developed countries to force them into policies of stagnation. These countries, led by Brazil, have voiced increasing scepticism of the United Nations' shifting priorities, from the traditional concerns with peace and development to such subjects as outer space, the seabed and the environment.

To counter the claim that the environment is of no concern to the developing countries until they become industrialized, that they should follow the tradition of the developed countries and not waste money on environmental problems until then, the conference planners have stressed the particular needs of the

developing nations in this area, calling this conference unique not only because it is the first global conference of its kind but also the first specifically to consider the problems of the developing nations.

Ambassador Olof Rydbeck of Sweden, at an informal meeting in November, said that "a basic aim of the conference . . . should in our view be to find appropriate and acceptable methods of harmoniously integrating environmental factors in the planning for economic and social development at the international, regional and national levels. By doing so, developing countries would be enabled both to avoid many costly mistakes already made in the industrialized countries and to cope with some of their serious existing environmental problems."

The developing nations themselves must, from this point of view, first indicate their own priorities, but the proposals already made include a fuller exchange of knowledge and experience, possibly through the establishment of one or several international information centres on the environment or by increased provisions for environmental education and training in the developing countries. Ambassador Rydbeck and others continue to stress that while proper planning must include environmental considerations, there is no reason to assume that higher costs will necessarily follow. They point out that the World Bank is beginning to include environmental costs in planning. The governments of Sweden and others hope that as a result of the conference, the cost of clean air and clean water will finally be considered as important as the cost of fuel.

The theme of the 1972 conference has been throughout the relationship between man and his environment. It is not seen as a technical conference devoted to

specialized papers but rather as an "action-oriented meeting" of scientists, planners and government officials to discuss a selective agenda that can lead to concrete actions. The hope is that the conference will identify those problems which can only, at best, be solved through international cooperation and agreement, promote a broad exchange of knowledge and experience between countries on means and methods for solving environmental problems, and encourage research by governments, international organizations and private institutions. The conference will also consider a possible Declaration on the Human Environment. An important though secondary result of the conference is seen as increasing the awareness of governments and public opinion on environmental problems—by the time the conference meets in June 1972, the environment may no longer be a public preoccupation.

Mr Strong proposes that the conference should be structured on three levels. The first is an intellectual and conceptual attempt to identify the major areas of consensus and the gaps in present knowledge, to identify priority issues and indicate the direction in which action should proceed. He hopes to have a working paper by next August. The next step will be to define a strong action programme that would make concrete recommendations and set guide-lines for the next ten years. Finally, there will be the publication of a few important items that could be acted on by the conference. These items will have to be identified during the meeting in February and the provisional list includes the establishment of regional and international monitoring networks, a global data bank for acquiring, storing, and exchanging data on

atmospheric and oceanic pollution, the prohibition at sea by international convention of oil pollution and possibly other harmful emission into the oceans and an international convention for the rational management of the resources of the seas.

BIOLOGICAL WARFARE

Detrick Left Hanging

by our Washington Correspondent

A YEAR and a month after President Nixon announced that stockpiles of biological weapons would be destroyed, the Army has discovered a foolproof way of doing so—by heat. Army spokesmen furnished elaborate explanations just a week before Christmas of the plans to steam heat the offensive germs and toxins, autoclave them and process the material through a sewage plant, and finally to bury the evaporate four inches below ground and plant grass above its grave. Apart from the last of these steps—a deft piece of symbolism indicating the care the Army is spending on its public relations on this occasion—the procedure seems hardly to differ from the standard methods in everyday use at the Army's biological warfare establishments. The inordinate delay in arriving at so plain a solution must be ascribed, by those who cannot believe the Army would try and obstruct the stated will of the President, to a truly massive bureaucratic inertia.

The nature of the biological weapons was not revealed at the Army press conference but they are believed to consist chiefly of anti-personnel agents, including the organisms or toxins of diseases such as tularaemia, Q fever, anthrax and Venezuelan equine encephalitis. These are stored in refrigerated igloos at Pine Bluff in Arkansas, along with an arsenal of 20,000 botulin charged bullets. Smaller amounts of anti-crop agents such as rice blast and wheat rust are kept at Fort Detrick in Maryland, the chief biological warfare research laboratory, at Rocky Mountain arsenal in Denver, Colorado, and at Beale air force base in Marysville, California. All agents are to be disposed of on site at the various storage locations, thus avoiding the troublesome public protests that are now a regular feature of the Army's schemes for transporting hazardous weapons across country. The cost of the condemned weapons, which were manufactured between 1962 and 1969, is \$726 million. The disposal plans will cost the taxpayer a further \$12.2 million.

Although these unpleasant organisms and their products have at last been consigned to certain extinction, a less certain fate awaits the Fort Detrick laboratories where most of the relevant research was done. Until recently, there seemed a good chance that the powerful research team at Fort Detrick, still numbering some 300 scientists and 700

supporting staff, would be kept intact and put to work on various health research projects under the authority of the National Institutes of Health. Both the scientific qualifications of the staff and the nature of the facilities would enable Fort Detrick to assume an immediately useful role, particularly in the handling and storage of dangerous viruses and viruses with long incubation periods. The necessary funds to convert Fort Detrick to a peaceful role—a mere \$15 million in a \$19,000 million bill—were agreed to by the Senate but struck out last month in the conference meeting with the House, which had not provided for Fort Detrick in its version of the bill. The conferees in their report said the proposed conversion should be further studied by officials of the Department of Health, Education and Welfare, who should be prepared to testify before Congressional Appropriations Committees next year. Although another study is not in itself a bad thing, the prospect of a further delay may prove too much for the Fort Detrick scientists whose future has been uncertain ever since President Nixon's renunciation of biological warfare over a year ago. The research team now seems likely to be disbanded as its members go looking for jobs elsewhere rather than face further uncertainty and delay.

SPONSORED RESEARCH

Harvard's Criteria

THE Faculty of Arts and Sciences at Harvard University at a meeting last month adopted six criteria for support of research within the university by outside agencies.

These six principles represent a codification of rules, both written and unwritten, followed since the end of World War II. The new code was recommended by the faculty's Committee on Research Policies and endorsed by the Faculty Council. The committee's report said:

"Our Committee subscribes to the principle of freedom of research as it has been traditionally interpreted in the university. This principle established the right of the scholar to determine the subject matter and sponsorship of his own research, and protects him from the imposition on his work of goals or criteria other than professional ones.

"In discussing the freedom of research it is important to distinguish between the methods and techniques of research on the one hand, and the subject matter or conclusions of the research on the other. The methods of research are clearly subject to limitations of a variety of sorts. For example, research techniques which might injure human health, or invade personal privacy without consent, or which involve unnecessary pain or suffering to any living things, are already

partially or wholly proscribed. Programmes which interfere with the freedom of research of other scholars by preempting space and facilities, or by involving the university in future commitments of its unrestricted funds, cannot be left entirely to the decisions of the scholar participating.

"In contrast with methods, however, the subject matter of research and the conclusions reached should be the sole responsibility of the scholar himself for which he is answerable to his scholarly peers only in his individual capacity. If this freedom sometimes results in research that some may regard as trivial, shoddy, or wrong, or in conclusions which some politicians misquote or misuse for purposes thought undesirable this is a risk which must be run, and it appears a lesser evil in comparison with allowing one segment of the academic community to impose its own standards of truth on another."

The text of the six points is as follows.

- (1) Any research agreement between the university and external sponsor must have obtained some form of sanction in advance. The purpose of this sanction is to ensure that the research conforms to the administrative and fiscal policies of the university, and to the present principle, and that it does not conflict with the rights of other scholars in the university or with other university commitments.
- (2) The source of sponsorship and the purpose of the research must be of such a nature that they can be publicly disclosed.
- (3) The university will not undertake to grant any exclusive information to a research sponsor, nor will it accept research which carries security classification, requires security clearance of university personnel, or otherwise precludes general publication of results.
- (4) All research projects must be undertaken with the clear understanding that the investigators concerned have the full right to publish any results obtained by them, subject to established safeguards for the protection of privacy, or confidentiality of personal data.
- (5) Any results obtained and any research published or lectures given by investigators on research projects are the sole responsibility of the investigator concerned, and Harvard University provides no institutional endorsement of the work or of the sponsor.
- (6) All the research on living animals and on human space should follow the safeguards established by the university for such work.

The committee also noted the criteria do not necessarily apply to the outside activities and to the consulting work of faculty members, which are subject to other policies of the university. "We have reviewed the document, statement of policy on conflicts of interest for the Faculty of Arts and Sciences, and we believe that it adequately covers the principles which should govern the outside activities of members of the academic community. We do suggest, however, that these principles be specifically brought to the attention of the officers."

NEWS AND VIEWS

Keeping Up to Date

THE pages which follow require some explanation. From today, *Nature* will be published in three editions each week, on Monday, Wednesday and Friday. The Monday edition, to be known as *Nature Physical Science*, is intended to be primarily of interest to scientists working in the physical sciences. The experience of the past few years suggests that the Monday edition will be strong on astronomy and plate tectonics, but the long-term intention is that it should give its readers a broad view of developments in all branches of physical science. Similarly, the Wednesday edition of *Nature*, known as *Nature New Biology*, will concentrate on topics primarily of interest to biologists. Inevitably, much of the original material to be published on Wednesday will consist of what is at present known as molecular biology, but here, too, the intention is to deal with all aspects of the biological sciences.

But is there not a danger that segregating original research into the Monday and Wednesday editions will rob *Nature* itself of its interdisciplinary character? This is an anxiety to be reckoned with, for the conventional and the frequently artificial lumping together of particular research into arbitrary categories has been a mixed blessing. Specialists, to be sure, are inconvenienced if they are enabled the more easily to find their way about the scientific literature and the popular complaints about the supposed jargon of special branches of science are not to be taken as seriously as they are intended—one of the great strengths of modern science is the facility with which new languages can be invented (and afterwards discarded) to describe novel sets of circumstances. Yet there are grounds for fearing, first of all, that too much segregation of specialized research deprives scientists in other fields of the intellectual stimulation that comes from following important developments elsewhere. Has not every professional scientist been stimulated by the remarkable way in which the whole of geophysics has been trans-

formed within a few years by the discovery of seafloor spreading and the evidence which it provides for the supposition that the crust of the Earth consists of movable and moving plates of material? If such intellectual revolutions are possible in staid branches of science such as geology, who can foresee where the next upheaval will come?

So important is the need for interdisciplinary communication that in the new arrangements for the publication of *Nature*, care will be taken to ensure that the Friday edition (this one), intended to be read as widely as possible by specialists as well as those with a general interest in science, will contain a wide selection from the original records of research submitted for publication. More rigorously than in the past, however, care will be taken to ensure that the material published on Friday is not merely of general interest but that it is accessible to a general audience as well.

Sometimes it will be necessary to negotiate with authors about the way in which their material is presented. Sometimes it will be preferable to publish the original paper on Monday or Wednesday and a summary of its conclusions on Friday. And as a matter of routine, the Friday edition will contain each week a number of short articles describing those of the contents of the Monday and Wednesday editions which seem to be of general interest. In the seven pages which follow, articles of this kind will be found in the bottom right-hand corner.

For the time being at least, it will be hard to predict just where these new developments will lead. Quite soon, however, it should become apparent that there is more scope within the framework of the new Friday edition for a wide and general coverage of the progress and the politics of science. But each of the three editions will be designed not merely as a laconic record of events but in the hope that it will be what every journal should be—a pleasure in its own right to read.

INFLUENZA VIRUS

Influenza to Order

from our Cell Biology Correspondent

WITH Christmas over and the influenza season as inexorably about to begin, the latest issue of *Virology* makes topical reading. No fewer than six papers are devoted to the biology of influenza viruses, and although none of them has great clinical implications, such a volume of research must be a crumb of comfort to those who seem annually to have to spend a few days in bed sweating out influenza.

Webster's work (*Virology*, 42, 633; 1970) has led to a new technique for producing to order influenza A viruses with particular desired combinations of the two virus-coded surface antigens—the haemagglutinin and the neuraminidase.

By growing strains of mammalian and avian influenza A viruses in pieces of chick allantois attached to fragments of shell viruses which are stable antigenic hybrids, viruses which have the haemagglutinin of one parent and the neuraminidase of the other can readily be selected from the mixed progeny. In essence, Webster uses combinations of antisera against either antigen of either parental strain to inactivate all but those recombinant particles carrying the antigens in the desired combination. Apart from facilitating studies of the immune response of an infected animal to particular influenza antigens in the absence of their natural partners and the chemical isolation of such antigens, Webster speculates that the ease with which antigenic hybrid viruses can be produced in the laboratory may throw light on the origins of human pandemic strains such as those which made the winters of 1947, 1957 and 1968 so miserable for so many. Perhaps recombinants between animal and human strains are the source of such pandemic viruses.

There are many conflicting reports in the literature about precisely how many species of polypeptides there are in an influenza virus particle, and the experiments of Haslam and her colleagues (*ibid.*, 555, 566) may go some way to clarifying the problem. They have analysed by polyacrylamide gel electrophoresis the polypeptides of particles of the A/Bel strain. Two glycoproteins and two arginine and methionine-rich polypeptides are resolved by this procedure.

The larger of the polypeptides (VP2) is the nucleocapsid protein and has a molecular weight of about 50,000. The other polypeptide (VP3), of unknown function, weighs about 21,000 daltons. The major glycoprotein (VP1), the haemagglutinin, weighs about 77,000 daltons; it is prone to disaggregation or breakdown and this may account in part at least for various conflicting reports of the number of "proteins" in an influenza particle. Furthermore, the electrophoretic mobility of this glycoprotein is variable depending on the nature of the host cell in which the virus was grown. This finding suggests that the carbohydrate moieties may be determined by the host cell while the protein is specified by the virus. In short, influenza particles seem to contain at least four and perhaps as many as six polypeptide chains, two of which are glycosylated.

When cells are infected at high multiplicities with influenza virus, varying proportions of the progeny virions are non-infectious and the proportion of non-infectious particles in a stock increases with serial passage. According to Meier-Ewart and Dimmock (*ibid.*, 794), the viral neuraminidase may function in the generation of non-infectious particles. By inactivating with specific antibody the neuraminidase of infecting viruses, they have strikingly reduced the proportion of non-infectious particles in the progeny yield. Meier-Ewart and Dimmock suggest that the generation of such particles depends on a balance in the infected cells between the amount of viral neuraminidase and cellular neuraminidase substrate. Tilting this balance in favour of the enzyme results, they argue, in increased production of non-infectious particles and vice versa, but how neuraminidase comes to affect the assembly of progeny influenza RNA genomes is as yet unknown.

It is well established that the genome of an infectious influenza virus comprises five pieces of RNA, the largest of which is missing from the genomes of non-infectious particles. Choppin and Pons (*ibid.*, 603) have now established, using the WSN strain of virus to infect a line of bovine kidney cells, that the formation of non-infectious particles with deleted genomes is enhanced by the presence of such particles in the infecting stock. When grown on the bovine cells, stocks of WSN virus contain very few non-infectious particles and several serial passages are required to obtain non-infectious particles from this host. Furthermore, HeLa cells infected with stocks of WSN grown on bovine kidney cells yield infectious progeny virus. On the other hand, HeLa cells infected with stocks of WSN grown on chicken cells, which contain many non-infectious particles, do not yield infectious progeny. Clearly the production of the defective particles depends on host as well as viral factors

and HeLa cells are not absolutely non-permissive for influenza virus.

RIBOSOMES

Streptomycin Binding

from our Nucleic Acids Correspondent

AN interesting experiment concerning the binding of ^3H -labelled dihydrostreptomycin to the digested ribosomal particles concludes the work of Chang and Flaks (*Proc. US Nat. Acad. Sci.*, 67, 1321; 1970) which I introduced last week. These authors found that six proteins can be removed without affecting the attachment of the antibiotic to the particle. But when proteins p8 and p11 (Nomura's nomenclature) are removed, the ability of the ribosomal particles to bind streptomycin decreases, suggesting that these proteins are directly or indirectly involved in the binding. This is surprising because protein p10 is known to be the determinant of the streptomycin sensitivity-resistance phenotype. This protein remains in the trypsin-resistant core particle and it must be concluded that several proteins have some influence on the single streptomycin-binding site within the 30S ribosomal subunit.

Although streptomycin binding and the

streptomycin resistance locus are frequently made use of in studies of ribosome structure and genetics, and protein synthesis generally, the *in vivo* action of the antibiotic remains unclear. It certainly causes extensive misreading of the genetic message by distorting the site at which the codon on a messenger RNA and the anti-codon on a tRNA pair (the A-site). But this is insufficient to explain the rapid and complete inhibition of protein synthesis *in vivo* occasioned by streptomycin. Modolell and Davis (*Proc. US Nat. Acad. Sci.*, 67, 1148; 1970) have studied this question further, and have concluded that the drug also acts on the puromycin-reactive (P) site, where peptidyl tRNA is transiently bound. They found that when formyl-methionine tRNA is bound to 30S ribosomal subunits in the presence of AUG, poly-AUG or R17 bacteriophage RNA, no inhibition results from the presence of streptomycin. On addition of 50S subunits, however, breakdown of the resulting initiation complex occurs. This breakdown is greatly accelerated by addition of puromycin, revealing that the formation of the complex as such is not interfered with, but rather that the stability of the complex is reduced. When the analogue GMPPCP is substituted for GTP, the fMet tRNA does not reach the site at which it is released by puromycin,

Ribosomal Synthesis with Ester Linkages

THE exploration of protein biosynthesis has traditionally been aided and abetted by clever tricks and artifices involving unphysiological situations. An important experiment in this genre is reported next week by Fahnestock and Rich (*Nature New Biology*, 229 8; 1971), who have demonstrated that during the formation of a peptide coded for by a natural messenger RNA, the peptidyl transferase of *Escherichia coli* ribosomes can catalyse the incorporation of α -hydroxy acids, rather than α -amino-acids, with ester instead of amide linkages.

Fahnestock and Rich seized on the recent finding that puromycin retains its antibiotic activity when its α -amino-group is replaced by a hydroxyl group. On reaction with formyl-methionine tRNA, an ester is formed. They wondered whether esters could be formed by the peptidyl transferase during true protein synthesis, and deployed the valuable system afforded by the R17 bacteriophage RNA containing an amber (UAG) terminating codon as the seventh triplet of the coat protein cistron. Normal translation of this sequence yields a hexapeptide of defined sequence (fMet-Ala-Ser-Asn-Phe-Thr). Instead of using aminoacyl tRNAs as substrates in this reaction, Fahnestock and Rich used in two cases the corresponding α -hydroxyacyl tRNAs by converting alanyl- and phenylalanyl tRNAs to lactyl- and

phenyllactyl tRNAs respectively (with nitrous acid treatment). If ester formation can indeed occur, these residues would be incorporated into the second and fifth positions within the peptide. Such ester linkages would be detectable by their susceptibility to milk alkaline hydrolysis (with molar triethylamine) which would leave the amide linkages unscathed. Fahnestock and Rich labelled the terminal formyl-methionine residue with ^{35}S , in the expectation that hydrolysis of a peptide containing an ester linkage at the second position would yield radioactive formyl methionine alone, and that hydrolysis of an ester at the fifth position would give rise to a radioactive tetrapeptide. The products were examined by electrophoresis in the presence of appropriate markers.

The results were gratifyingly clear, showing that the α -hydroxy acids were indeed incorporated into the correct internal positions in the peptide, although the peptidyl transferase was not entirely fooled because the yield of the hexapeptide was markedly reduced when the hydroxyacyl tRNAs were substituted for the aminoacyl tRNAs. Fahnestock and Rich make the interesting suggestion that because the peptidyl transferase can manifestly use either the nitrogen or oxygen atom as a nucleophile, it could equally catalyse the hydrolysis reaction known to occur in chain termination.

and is stable in the presence of streptomycin. Breakdown only occurs when the initiation complex is completed on the fMet tRNA entering the P site. Modolell and Davis also believe that the ribosomes released as a result of the breakdown of the abortive initiation complex may not be able to form further initiation complexes, although the reason for this is still unclear.

ANTIBODIES

Variations on a Theme

from our Molecular Biology Correspondent

EVEN such readers as have to make a conscious effort to recollect which is the antibody and which the antigen should find some interest in a number of elegant new uses to which antibodies can be put. Melchers and Messer (*Europ. J. Biochem.*, **17**, 267; 1970), for example, have developed a method, first contrived by Rotman and Celada, for the detection and isolation of defective enzymes produced by mutant cells. The β -galactosidase of *Escherichia coli* remains active when associated with its specific antibody, and can be caught on a column of 'Sephadex' to which the antibody has been covalently attached by methods that are now standard. Melchers and Messer have screened a series of *E. coli* mutants containing defective β -galactosidase, which in the purified state is essentially inactive. It is activated, however, by the antibodies to a level approaching the activity of the wild type enzyme. This is a remarkable though not unique phenomenon, the explanation of which is by no means obvious. Inasmuch as the antibodies recognize, bind to, and therefore stabilize the native conformation of the enzyme, one may envisage that a molecule containing an unfavourable amino-acid substitution that destabilizes the native conformation may still be trapped in that state in the antibody complex.

At all events, the mutant enzymes all cross-react with the antiserum to the wild type, and can be recognized in their antibody complexes by the appearance of the latent enzymic activity. After adsorption on the columns, the antigen-antibody complexes can be dissociated, and the enzyme recovered, by acid, urea or increased temperature. The molecular weights of the mutant β -galactosidases isolated in this way were in all cases the same as that of the wild type, and the defects therefore neither bring about nor are caused by failures in quaternary association. This method of extracting mutant proteins could clearly be of great value, especially perhaps when they are inactive, and cannot be easily identified. It is necessary only that the antigenic determinants on the native protein should be present in the mutant, and that the wild type antigen be pure in the first place. It is also worth observing that

when an antigen has an assayable enzymic activity, which is preserved in its complexes with antibody, the use of enzymic assay offers an amplification mechanism, whereby otherwise undetectably small amounts of the antigen can be determined.

A pretty variation on the same theme has been developed in Sela's laboratory, and involves chemical coupling of antigens to the surface of a bacteriophage. On addition of antibodies the phage is inactivated, and because of the sensitivity of assays of phage infectivity, minuscule amounts of antigen and of antibody can be detected by this means. Hurwitz, Dietrich and Sela (*ibid.*, 273) have now applied this strategy to the detection of an angiotensin. This is a peptide hormone, only fourteen residues long, and, like other such small molecules, exceedingly difficult to detect by ordinary means, for its complexes with antibodies do not precipitate. Antibodies can be produced by coupling the angiotensin as a hapten to a carrier protein. Coupling of the peptide to T4 bacteriophage by way of a bifunctional

reagent, glutaraldehyde, presents no difficulties. The specific antibodies can be stripped out of the antiserum if required by passage through a column of angiotensin bound to 'Sephadex'. With unfractionated antiserum, however, very small antibody titres can be detected by the phage assay. Moreover, the phage inactivation is quantitatively inhibited by free angiotensin, and the most important upshot of this work therefore is that it allows the detection of as little as 10 pg or less of free angiotensin.

Another recent example of the use of specific antibodies is in the removal of a contaminant enzyme from the plant protease, phaseolain (Carey and Wells, *Biochem. Biophys. Res. Commun.*, **41**, 574; 1970). The contaminant, which is an endopeptidase, can be prepared in pure form more readily than it can be quantitatively removed from the phaseolain. It can therefore be used to stimulate antibodies, and phaseolain preparations extracted with the antiserum. The phaseolain could then be characterized as a true carboxypeptidase with no endopeptidase activity of its own.

Processing of tRNA Precursors

It is well established that mature ribosomal RNA in bacteria arises by the processing of larger precursor molecules. Altman has now isolated tRNA precursors and he describes his method in next week's *Nature New Biology* (**229**, 19; 1971).

He took advantage of the invaluable system developed by J. D. Smith and his colleagues whereby the gene for tyrosine tRNA (either wild type or amber suppressing), or various mutants of this gene, can be transduced into *Escherichia coli* with the aid of the bacteriophage $\phi 80$. Large amounts of this tRNA are then synthesized in the post-infection period, in the wild type and suppressor tRNAs. Altman examined two mutants in which the tyrosine tRNA was synthesized at a much lower rate after infection. He found that when short pulses of ^{32}P (3.5 min) were administered to the infected cells, and the newly synthesized, labelled RNA was rapidly extracted and fractionated by polyacrylamide gel electrophoresis, a new prominent band appeared which was not present in extracts of cells infected with phage carrying the wild type or suppressor genes. From its mobility during gel electrophoresis, this material contains about 200 nucleotides. Fingerprints of this band revealed that all the oligonucleotides of the tyrosine tRNA were present within it, with the exception of the products from the 3' and 5' termini. There were also a number of additional products, among them an oligonucleotide which may be the 3' terminal fragment without the $-\text{CCA}_{\text{OH}}$ terminus.

Altman suggests that this material is a precursor of the tyrosine tRNA, and he proposes that it is accumulated to a greater extent during short labelling times in the cases of the mutants (with consequently diminished amounts of mature tyrosine tRNA), because the processing of the precursor is less efficient than with the wild type or suppressor strains. This is confirmed by the finding that an additional band can also be isolated when cells infected with phage carrying the wild type or suppressor genes are pulse labelled at 25° C. This band (about 140 nucleotides or so in length) also contains the set of the tyrosine tRNA sequences. Presumably the processing of the larger precursor molecules is carried out more slowly at this temperature. Measurements of the half-lives of these precursors indicate a value of about 3 min for the mutant strains at 37° C and for the wild type or suppressor strains at 25° C.

The two mutants I have described were complex. But a further mutant with only a single defined nucleotide change also accumulated a precursor, slightly smaller than that accumulated with the wild type. This surprising result implies that the "tailoring" enzymes are sensitive to small changes even when well removed from the termini of the tRNA. All the precursors exhibited certain spots in common, in addition to the products from the tRNA itself. These other products totalled about forty nucleotides. This suggests that at least the later part of the biosynthetic process is likely to be common to all of them.

NUCLEIC ACIDS

No New Interactions

from our Molecular Biology Correspondent

THE organizers of the Christmas meeting of the British Biophysical Society in London on December 14 and 15, made an prising attempt at the impossible, and failed, in the sense that no general picture ever took shape, and no rules concerning the nature of nucleic acid-protein interactions in different circumstances were formulated. The meeting was therefore no more than a collection of talks falling loosely into three or four groups, though in themselves they maintained a high level of interest.

There were two papers on the interaction of nucleases with their substrates. F. M. Richards (Yale) gave a comparative account of the X-ray structures at atomic resolution of two nucleases and their complexes with inhibitors—his own RNase S and Cotton's staphylococcal nuclease. In the former case there are now some reasonably well-established ideas about the mechanism, and the interaction of the active centre residues with the nucleotide, involving a considerable displacement of one histidine, can be observed in detail. In staphylococcal nuclease the situation is relatively obscure, and there is a strange dearth of residues generally regarded as catalytically interesting (notably histidine) in contact with the substrate. Two tyrosines must be presumed to be implicated in the binding. Richards pointed out that a very large displacement of a phenolic ring (also found to occur in carboxypeptidase on binding of inhibitor) results in fact from a rotation about one C-C bond, which is structurally a very small event. No general conclusions at all are available about the nature of nucleotide binding sites. G. C. K. Roberts (Cambridge) showed the extraordinary degree of stereochemical detail that can be obtained from high-resolution NMR studies, with particular reference to interaction of pancreatic ribonuclease with inhibitors.

L. Lerman (Vanderbilt) offered evidence of a new form of DNA, the ψ -structure, which appears in solutions containing high concentrations of neutral or acidic polymers together with salts. The conditions were selected to simulate more closely the dielectric environment *in vivo*. Lerman showed that under critically defined conditions an optical and hydrodynamic transition occurs to a form characterized by compact folding and unusual circular dichroism. Strenuous attempts were made to exclude aggregation effects and association with the added polymers. The unusual spectroscopic properties of DNA in phage heads can in principle be explained by the presence of the ψ -conformation. There will be some argument about how well the data can be reconciled with

published work by Maestre and others.

The work of M. Nomura (Wisconsin) has provided the most important new results in the study of ribosomes in the past few years. At the meeting, Nomura described the details, with assembly maps, showing the relative roles of different proteins, of the reconstitution of active bacterial 50S ribosomes. A new departure in his work concerns the function of the colicin factor that kills *E. coli* by inhibiting ribosomes. Recombination experiments with components from colicin-inactivated and normal cells showed that colicin operates on the ribosomal RNA, not, like other antibiotics, on the proteins, and it turns out that the RNA of the 30S particle in fact loses nucleotides from its 3' end. Such a slightly truncated RNA will combine with proteins to regenerate an inactive particle. It differs from intact RNA in that one protein fails to bind, and it was established that this component (P15) is essential to the function of 30S ribo-

somes. The essential nature of the 3' terminus is also consistent with the inactivity of 30S ribosomes made with the 16S precursor RNA, which is a little longer at both ends. Both in this and the RNA from colicin-treated cells, a dimethyladenine seems to be missing from the RNA. Nomura also described the isolation of cold-sensitive mutants, which did not grow at 20° C, and then containing only partly unfolded ribosomal subunits just as when *in vitro* the heating step is omitted.

J.-P. Waller (Gif-sur-Yvette) presented results showing that methionyl-tRNA synthetase on proteolysis dissociates from a tetramer to an active dimer, with loss of an 11,000 molecular weight fragment. He suggests that this is, in effect, a separate protein, covalently associated with the rest possibly in consequence of gene fusion, and that it directs the formation of the correct quaternary structure. It is not yet clear why the tetrameric state should be advantageous.

Sweeping out Double Stranded RNA

THE idea that the single stranded RNA genomes of the RNA tumour viruses may be replicated independently of the synthesis of a complementary DNA molecule is rendered very unlikely by the work of K. K. Reddi to be published in next Wednesday's *Nature New Biology*.

RNA viruses live in a world dominated by DNA; the genetic material of the cells which they parasitize is after all DNA, and as far as is known cellular RNA molecules function only as intermediaries in the expression of DNA genes. The host cell has all the enzymatic machinery required for the self-replication of DNA but presumably it lacks enzymes which can replicate RNA because it has no use for them. The evolution of the RNA viruses has therefore depended on the emergence of some mechanism for the faithful replication of RNA molecules.

On the face of things there are only two alternative pathways for the replication of an RNA genome—either the RNA can be self-replicated quite independently of any DNA metabolism, or the viral RNA genome might be copied into DNA which could then be replicated in the same way as the cell DNA and also act as a template for the synthesis of progeny RNA molecules. Apparently the double stranded RNA viruses and most of the single stranded RNA viruses, including the RNA bacteriophages, polio virus and its relatives, the influenza viruses and the parainfluenza viruses have evolved along the first of these paths. They seem to specify an enzyme or enzymes which can replicate RNA directly. And of course in the case of the single stranded RNA viruses this replication involves the conversion of the input single strand into a double stranded RNA molecule by the

synthesis of an RNA complementary to that in the virion which infects the cell.

The RNA tumour viruses and a few other single stranded RNA viruses, by contrast, have evolved along the second path. As Temin and Baltimore proved last year, the replication of these viruses involves the synthesis of a double stranded viral DNA from the input single stranded viral RNA. But might not these viral RNAs be self-replicated as well as replicated by way of a DNA? There is no reason *a priori* why this should not happen, and early last year Biswal and Benyesh-Melnick in Texas and Griensven and his colleagues in France claimed to have discovered a double stranded RNA in mouse cells infected and transformed by mouse leukaemia and sarcoma viruses.

Attempts to repeat these observations have, however, failed. K. K. Reddi now reports experiments which drive another nail into the coffin of the idea that RNA tumour viruses can replicate other than by way of a DNA intermediary. In short, Reddi has searched for a double stranded RNA in chick cells transformed by Rous sarcoma virus, taking care to use methods which do not generate artefactually double stranded RNAs. The only double stranded nucleic acid he can detect is DNA, not RNA. As he says, "evidence for the existence of ribonuclease-resistant RNA is lacking", and only double stranded RNA is ribonuclease resistant. Negative results are notoriously difficult to interpret in a meaningful way but, with the odds stacked so high against it, the suggestion that the RNA genomes of RNA tumour viruses can replicate independently of a DNA intermediate is probably best forgotten.

PHOTOSYNTHESIS

Parallel Photosystems

from a Correspondent

THE process by which green plants capture the energy of the Sun and convert it into chemical energy stored in the form of carbohydrates may involve three separate light reactions, and not two as previously thought.

In photosynthesis, water is split into oxygen and hydrogen ions by light energy from the Sun, and the resulting electrons are used to reduce carbon dioxide to carbohydrate. So far, the most popular model for explaining this transfer of electrons to carbon dioxide involves two separate light reactions acting in series (see D. A. Walker, *Nature*, **226**, 1204; 1970). Photosystem two (S2) is associated with the oxidation of water and the concomitant evolution of oxygen. Photosystem one (S1) accepts electrons from the primary photoreductant of S2 by way of electron carriers and promotes them, by a second light reaction, to the redox level of ferredoxin, an iron containing protein, and thence by enzymic reaction to nicotinamide adenine dinucleotide phosphate (NADP).

Arnon and his colleagues have, since the mid-sixties, considered this scheme to be incorrect and have argued that the two photosystems (S1 and S2) act in parallel rather than in series, when bringing about carbon fixation (*Nature*, **207**, 1367; 1965). They have presented evidence that S2 alone is capable of reducing NADP and that the function of S1 is to produce adenosine triphosphate (ATP) by cycling electrons through a system of linked redox carriers. According to the Benson-Calvin scheme for carbon fixation in photosynthesis, both NADPH₂ and ATP generated in the light are required for converting carbon dioxide into carbohydrate (D. A. Walker, *Nature*, **226**, 1204; 1970). From the energetic point the parallel scheme did not seem entirely satisfactory, for it required that only one photon of red light was available to raise an electron from water to NADP.

In a recent article from Arnon's laboratory (*Proc. US Nat. Acad. Sci.*, **67**, 1404; 1970) additional evidence is presented for the parallel scheme and it suggests that S2 consists of two photo-reactions S2a and S2b. These two photo-reactions are thought to act in series linked by a chain of electron carriers including cytochrome *b*₅₅₉ and the copper containing protein plastocyanin. Photoreaction S2b finally brings about the reduction of NADP by donating its excited electron to ferredoxin. The evidence of Arnon *et al.* for this new scheme comes from studies of fragments of spinach chloroplasts rich in either S2 or S1 activity. The S2 particles were able to photo-oxidase cytochrome *b*₅₅₉ and, moreover, could in the presence of plasto-

cyanin bring about a light induced evolution of oxygen from water and a reduction of ferredoxin and hence NADP. In further agreement with their model they found no evidence that S1 mediated reactions such as the oxidation of the S1 trap (P700) or its associated cytochrome (cytochrome *f*) were involved in the photoreduction of NADP with water.

Although Arnon has been able to place his parallel scheme on a firmer basis energetically by introducing a third photochemical act, the model still does not explain many of the well established observations of other laboratories. For example, it is difficult to reconcile the parallel scheme with the work of Duysens and Ames (*Biochim. Biophys. Acta*, **64**, 243; 1970) who reduced and oxidized cyt. *f* by using light which selectively excited S2 or S1 respectively. Nevertheless, if the claim that S2 can operate independently of S1 in reducing NADP is correct then there is undoubtedly a need to modify the well established series formulation of electron transport in photosynthesis. Only time will tell if the model presented by Arnon and his col-

leagues will be a satisfactory modification.

MARINE FISH

Galway Plankton

from our Marine Vertebrate Correspondent

STUDIES of the planktonic stages of marine fish have been a feature of fisheries research in the North Atlantic for many years, but they have usually been carried out with a view to tracing the early stages of fishes of direct commercial importance. Detailed local studies of the plankton, such as those conducted by the Plymouth laboratory of the Marine Biological Association, are much fewer but they have considerable relevance in that they present an overall picture of the fauna in the surface layers of the sea. An intensive survey of the plankton in Galway Bay and of the west coast of Ireland has been conducted by the staff of the Department of Zoology, University College, Galway, of which the fourth part on the larval and post-larval stages of fishes taken in the plankton be-

Structure and Migration of the Solvated Electron

THE reactions of the solvated electron are the subject of two articles in next Monday's *Nature Physical Science*.

The study of the solvated electron has its origins in the work begun by Kraus at the beginning of this century on the blue solutions formed by dissolving alkali metals in liquid ammonia and amines. Electrical conductivity and other measurements have established that the solvated electron is responsible for the blue colour of these solutions, but, although Platzman predicted some of the properties of the hydrated electron in 1953, it is only in the past decade that the solvation of electrons by polar solvents has been generally recognized.

Reactions of the electron can now be measured routinely, but its structure remains uncertain. Several theoretical models have been developed all of which are quantitatively unsuccessful, particularly in predicting the asymmetry of the optical absorption band. Qualitatively the electron is usually regarded as occupying a cavity in the liquid in which the surrounding molecules are polarized, thus creating a potential well, but it is uncertain whether these vacancies pre-exist or are created by the electron. Work on electrons trapped in viscous super-cooled liquids and glasses supports the former alternative. Furthermore, the absorption band of these trapped electrons can be selectively bleached, by exposure to the appropriate wavelength light, showing that it consists of several overlapping bands which arise through the electron occupying vacancies of different sizes.

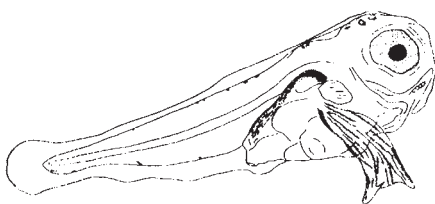
Catterall (*Nature Physical Science*,

229, 10; 1971) now suggests that the width of the absorption of the solvated electron in dilute metal-ammonia solutions may also be explained by the occupation of cavities having a (Gaussian) distribution of sizes. From studies of relaxation rates in magnetic resonance experiments Catterall concludes that the electron occupies small vacancies in the liquid, and that the large volume expansion resulting from dissolution of metals in ammonia arises through breakdown of the normal liquid structure surrounding the trapped electron rather than through the creation of large vacancies.

The magnitude and temperature dependence of the diffusion constant of the solvated electron are also important because they indicate whether or not the solvated electron diffuses like a normal solvated ion. Conductivity measurements (Barker *et al.*, *Trans. Faraday Soc.*, **66**, 1498; 1970) show the electron to have a diffusion constant of 4.8×10^{-5} cm² s⁻¹ at 25°C and an activation energy for diffusion of 20 kJ mol⁻¹. In contrast, Cercek reports (*Nature Physical Science*, **229**, 12; 1971) a value of 8.4 kJ mol⁻¹, which is about half that for the self-diffusion of water, and suggests the electron does not diffuse normally. His value is calculated from the activation energies of the reactions of the hydrated electron with a number of solutes having values in the range 9 to 11 kJ mol⁻¹, and assumes these are caused only by diffusion. Logan (*Trans. Faraday Soc.*, **63**, 1712; 1967) has, however, shown that the lowest measured activation energy cannot always be equated with that of the diffusion process.

tween 1958 and 1966 has just been published (J. M. Fives, *Proc. Roy. Irish Acad.*, 70 (B), 15; 1970).

Fives has found larvae or post-larvae of sixty-eight species of fish in these samples. Thirty-nine of these have not been recognized before in the plankton of Irish waters, but because few of them are rare or unexpected fish in this region this can only be a reflexion of the lack of studies of this type in Ireland. Most of these species are the smaller non-commercial species of fish, but it is surprising to find that the larvae of pilchard and sprat have not been recognized before, for these fish are abundant off these coasts. Fives's work has considerable potential significance to the fisheries for these species, for she gives details of the seasonal variation in numbers of larvae.



Post-larval stage of three-bearded rockling, *Gaidropsarus vulgaris*, from the plankton of Killeany Bay on the west coast of Ireland. Length 5.5 mm.

Sprat larvae, for example, were found from January to June, implying a long breeding season, although Fives suggests this may be explained by delayed larval development with low salinity in some areas. Pilchard larvae, however, are not found continuously during any one season, and their occurrence can be linked with large catches of *Sagitta elegans* (the planktonic arrow worm typical of water masses from the open Atlantic). It seems therefore that west of Ireland the pilchard spawns on off-shore banks and its eggs or larvae occur near the coast only through drift in water masses.

Several of the larvae recognized in the Galway survey seem not to have been described previously. Among these is the large sandeel *Hyperoplus immaculatus*, and the smooth sandeel *Gymnammodites semisquamatus*, both abundant fish, and, with their relatives, important links in the food web of the sea. Another interesting series of larvae is referred by Fives to the blenny genus *Coryphoblennius*. Although the young of the only Irish *Coryphoblennius* species (Montagu's blenny, *C. galerita*) are well known, Fives's description of the larvae seems to be the first. Fives's twenty-two specimens measured between 4 and 8 mm and were caught between June 30 and July 13.

This study of the larval planktonic fishes of Galway Bay has thus added something to general knowledge in the description and identification of a number of larvae which had previously escaped

notice. Its bearing on the spawning season and productivity of a number of commercially important fishes is considerable, and taken with the other studies on the zooplankton of the area it represents a contribution to the better understanding of inshore plankton.

CONTINENTAL DRIFT

Forceful Motion

from our Geomagnetism Correspondent
THE chief obstacle to the complete credibility of continental drift is, and always has been, the nature of the forces which drive the continents along. Before about 1950 the evidence for continental drift was more or less circumstantial; and this, combined with ignorance of a reasonable driving mechanism, produced little support for the hypothesis. During the past two decades, however, direct evidence for drift and seafloor spreading has proved utterly convincing; and conventional wisdom has adopted convection currents in the mantle as the *modus operandi* for both. But the direct evidence in favour of mantle convection remains nebulous.

Jacoby's new mechanism for drift, spreading and plate movements (*J. Geophys. Res.*, 75, 5671; 1970) is thus of considerable interest, and seems to be as convincing, if not more so, than convection currents. Jacoby suggests that the plates are not dragged along passively by the mantle but that both the lithosphere and asthenosphere actively participate in the movements as the result of gravitational instability in the upper mantle. More specifically, the active diapirism of the asthenosphere under the mid-oceanic ridges and the sinking of the lithosphere under the island arcs each exerts forces on the plates which tend to produce motion away from the ridges. Ultimately, the plates are driven by temperature-induced density contrasts between lithosphere and asthenosphere. The motions could, of course, still be regarded as the result of

convection currents; but the convection is vastly different from upper mantle convection as normally conceived.

But is such a mechanism feasible? Jacoby goes to great lengths to show that it is, using order of magnitude calculations and making many, but reasonable, assumptions. The first process he considers is diapirism under the ridges, by which he simply means the filling of the space between the spreading ridges as the mantle material rises and solidifies. Such diapirism requires, of course, an instability in the mass distribution of the crust and mantle under the ridges—a density reversal with depth, for example—which almost certainly exists. As the asthenosphere then rises diapirically it will lift up the lithospheric plates on either side of the ridge and the plates will tend to slide down away from the ridge under the force of gravity. Second, as the lithosphere descends under island arcs it will, unless the descending part is uncoupled from the rest of the lithosphere by vertical faults, exert a downward pull on the plate.

Against these driving forces must be weighed the resistances to plate movements. Material must, for example, be returned through the asthenosphere to the ridges against gravitational forces and against the viscosity of the asthenosphere itself. It is, of course, quite impossible to calculate accurately the balance between driving and resistive forces; but Jacoby's order of magnitude calculations do show that a net positive driving force is plausible. The energy to maintain this driving force would, presumably, be heat.

The model is, as Jacoby admits, greatly simplified. But it does not seem to conflict with the canons of continental drift, seafloor spreading and plate tectonics. Moreover, it has the advantage that some aspects can, in principle, be tested experimentally—for example, the necessary ridge structure, the gravity fields associated with various parts of the motions and the stress fields in the plates.

Excitation of 6300 Å Airglow

THE most naive view of the airglow used to be to regard it as a faint diffuse aurora, which might be excited by electrons. More recently, however, it has been widely accepted that the principal mechanism exciting 6300 Å was the dissociative recombination of O_2^+ ions, but now D. G. Nichol (*Nature Physical Science*, 229, 13; 1971) has produced strong evidence that excitation by electrons is important at high latitudes. Observations at Hobart, Tasmania (invariant latitude 54° S), show the airglow intensity increasing to the south (higher latitudes) whereas the *F*-layer electron density increases to the north. Nichol also

remarks on the frequent observation of simultaneous brightening of airglow from invariant latitude 50° to 65°. He finds that a flux of $\sim 10^{10}$ eV cm⁻² s⁻¹ could account for the airglow and suggests that electrons of 10–30 eV are responsible; measurements at these energies are, however, difficult. The airglow results are similar to those of B. P. Sandford in New Zealand (*Planet. Space Sci.*, 10, 195; 1964). Theoretically a drizzle of particles over a wide range of energy should be associated with convection in the magnetosphere, and would be expected to extend from the auroral oval to the plasmapause.

GEOPHYSICS

Biscay's Opening

from a Correspondent

A SYMPOSIUM on the structural history of the Bay of Biscay jointly organized by the Institut Français du Pétrole (IFP) and the Centre Nationale pour l'Exploration des Océans (CNEXO) and held at Rueil Malmaison near Paris, from December 14 to 16, was the first to be held specifically about the bay. The excellent communications between academic geologists and geophysicists and those from commercial companies were both welcome and valuable. Of the 200 or so delegates from nine countries, about 30 per cent were from commercial companies.

Since Carey's suggestion in 1958, various subsequent fits of the Atlantic continents, and the realization of the magnetic lineations in the Bay of Biscay, it has been fairly convincingly established that Spain has rotated in an anticlockwise direction away from France, thus forming the Bay of Biscay. The chief problems considered at the symposium were: when did the rotation take place and was the movement purely rotational or was there a combination of translational movement with rotation?

The several contributions on the geology of the Aquitaine Basin presented by representatives of oil companies indicated the extent to which duplication of work takes place. Notably, however, Dr E. Winnock (Société Nationale des Pétroles d'Aquitaine), who spoke on the Aquitaine Basin, and Dr R. A. Dardel (Esso), who spoke on the Parentis Basin, showed the basins to have evolved during the Trias. Two principal periods of disturbance in the Lower Cretaceous and Lower Eocene were followed by periods of less disturbed transgressive sedimentation. East-west faulting during the Jurassic and Cretaceous, coupled with tilting of the basins, indicates a subsiding continental margin while Biscay was opening.

Work on Pyreneo-Cantabrian structures by P. Feuille and P. Rat (University of Dijon) showed similar disturbances and a major palaeogeographic change at the end of the Albian. M. Mattauer and M. Seguret (University of Montpellier), from evidence of Triassic basin formation, however, concluded that opening of Biscay started in early Trias. Excellent work by G. Boillot *et al.* (University of Rennes, Rouen, CNEXO) showed that Pyrenean geology could be traced offshore along the north coast of Spain as far as Cape Ortegal.

At last petrologists working in north-west Spain and Brittany have correlated the geology of the two areas. This was very adequately shown by J. P. Bard, R. Capdevila and P. Matte (University of Montpellier), illustrating the similarities

between the granites, stratigraphy and the metamorphic zones of the two areas.

Studies of the floor of the Bay of Biscay, principally from reflexion profiles, showed that Cantabria and Biscay seamounts are both blocks of seafloor uplifted during the Eocene (P. Muraour *et al.*, University of Bordeaux). J. Ewing, L. Burkle and T. Saito (Columbia University) included Gascony seamount in the similarity and identified the three as being part of a linear east-west dislocation across the Bay. J. C. Sibuet, G. Pautot, Dr X. le Pichon (CNEXO) saw dissimilarities in sedimentation between north and south of approximately this line. JOIDES drill sites Nos. 118 and 119 (Dr A. S. Laughton, National Institute of Oceanography) have produced Palaeocene sediments, the oldest so far sampled. But seismic reflexion records of several authors all showed the disturbed Lower Cretaceous strata, followed by the less disturbed sediments of the Upper Cretaceous, similar to the geology of the same period on land.

The palaeomagnetic data of Drs R. van der Voo and J. D. A. Zijdeveld (State University of Utrecht) require that Biscay opened during the Late Trias to Late Cretaceous, but Dr K. W. Stauffer (American Overseas Petroleum (Spain))

and Dr D. Tarling (University of Newcastle upon Tyne) presented some new measurements which constrained the time of opening to between the Kimmeridgian and the Maestrician. The relationship between Atlantic and Biscay magnetic lineations (Miss C. A. Williams, University of Cambridge) suggests the opening was Lower Cretaceous or earlier.

The highlight of the meeting was the last afternoon with four contributions on structural hypothesis on the formation of the Bay of Biscay. Drs J. C. Sibuet, G. Pautot and X. le Pichon (CNEXO) put forward their ideas based on rotation about a centre located near Paris. The North Pyrenean Fault and faults crossing the Armorican and Spanish continental shelves, forming in some cases major features such as the Santander Canyon, were interpreted as being sub-parallel to circles about the rotation centre. Drs M. Bacon and F. Gray (University of Cambridge), on the other hand, required rotation and east-west translation along the line of the north Spanish trough.

No meeting, however excellent, can hope to solve all the problems. A considerable amount was achieved, however, in that it is now agreed that Biscay has oceanic crust in the centre with down-faulted continental crust along the edges and there is considerable agreement on the time of opening.

Barometers detect Earthquakes

OSCILLATORY motions of the atmosphere have been attracting a good deal of attention in the past few years. Most people would trace the resurgence of interest to C. O. Hines, whose missionary work in the early sixties for the view that many oscillatory effects in the atmosphere can be attributed to what are known as internal gravity waves is at the basis of much modern work. The measured data go back much farther, however, to what might be called the prehistory of atmospheric physics and such observations as the pressure pulse following the Krakatoa eruption. Recently, some geophysicists on the seismology side have also been developing an interest in the effect of such cataclysmic events on the atmosphere as a means of studying the degree of coupling between the Earth and the atmosphere, and vice versa. A coupling between large earthquakes and the atmosphere has already been reported in *Nature* (202, 1095; 1964) by B. A. Bolt, who detected an effect in barogram records at Berkeley following the severe Alaskan earthquake of March 28, 1964.

A similar report dealing with the energy transfer between the atmosphere and the Earth will be published in next week's *Nature Physical Science* by G. G. Sorrells, J. A. McDonald and E. T. Herrin of the Dallas Geophysical Laboratory (229, 14; 1971). Their equipment

is a triangular array of microbarographs in east Texas and two seismographs at the same location, one 183 m down a salt mine and the other at the surface. The use of an array of microbarographs means that the direction of arrival of the atmospheric signal can be determined in much the same way as the angle of arrival of radio waves is determined by ionosphericists using spaced receivers, and sufficient care was taken with the seismographs to ensure that they could not be triggered to any extent by pressure variations in the atmosphere. Having one seismograph down a mine helps here because the shaft acts as a filter between the instrument and the surface. Even so, on July 4, 1970, the seismographs registered a disturbance during the passage of an acoustic wave in the atmosphere, and having taken these precautions the group at Dallas believe that the seismographs were indeed recording a true motion of the Earth. They point out that the measurements agree with unpublished calculations by Sorrells of the response of the Earth to a plane pressure wave moving at an acoustic velocity. The degree of coupling is indeed very small, but the figures given by Sorrells indicate that a pressure wave moving at 330 m s⁻¹ generates a displacement of the Earth's surface of 10 to 15 nanometres per microbar with periods between 20 and 100 s.

EARTH'S MANTLE

New Peridotite Layer?

from our Geomagnetism Correspondent

ALTHOUGH the correspondence between mid-oceanic ridges and continental rift valleys may not be as close as is often assumed, the rift valley comes to mind when the landward extension of the oceanic ridge system is considered. The case for a genetic connexion between oceanic ridges and alpine complexes, on the other hand, is less clear, but is beginning to be heard more and more. The latest proponents of this idea are Bonatti *et al.* (*Earth Planet. Sci. Lett.*, 9, 247; 1970) who back their arguments with sound petrological and geochemical data from samples dredged from the equatorial mid-Atlantic Ridge. To be more specific, most of the samples originated in the region between 2° N and 2° S where the ridge is intersected by major fractures such as the Chain, the Romanche and the St Paul—in other words, where thick crustal sections are exposed and thus where normally inaccessible deep-seated material can readily be recovered.

What emerges from the petrological analysis of these samples—to take the Romanche fracture zone as a case in point—is an approximate stratigraphy for the mid-Atlantic Ridge, at least in this particular locality. Basalt, up to 2 km thick, grades downwards into gabbro and metamorphic rocks which overlie peridotites. This profile is remarkably similar to those of the classical alpine complexes—a similarity which is all the more striking because it is not customary to speak of oceanic ridges and alpine complexes in the same breath.

That this stratigraphic similarity is not merely an unfortunate coincidence is supported by the strontium geochemistry of the dredged samples. For example, whereas the basalts and gabbros have low $^{87}\text{Sr}/^{86}\text{Sr}$ ratios within the range previously obtained for oceanic basalts, the ultramafic rocks have ratios between 0.706 and 0.723, outside the normal basalt-gabbro range. This is also true for alpine peridotites but not, for example, for peridotite inclusions in basalt nor for peridotites from stratiform sheet complexes. It was the high ratios in alpine ultramafics which some years ago led Strueber and Murthy (*Geochim. Cosmochim. Acta*, 30, 1243; 1966) to the conclusion that alpine peridotites are residual, having been depleted in lithophile elements at some early stage in their history. And as a result of their own $^{87}\text{Sr}/^{86}\text{Sr}$ measurements, Bonatti *et al.* draw a precisely similar conclusion for the mid-Atlantic Ridge peridotites.

It would seem to follow from this that the mid-Atlantic Ridge basalts and gabbros are genetically unrelated to the peridotites which they overlie—which

means to say that the peridotites are neither the parent material for the basalts nor the residual product after their extraction. What, then, is the origin of the oceanic peridotites? Bonatti *et al.* suggest that they must have been emplaced as solid intrusions from below, which, in turn, must mean that alpine-type peridotite is a common constituent of the oceanic upper mantle, possibly as a continuous layer immediately below the crust. The course of oceanic basalt would then be the mantle below the alpine-type layer.

Assuming that this layer really exists, Bonatti *et al.* go on to speculate that the alpine-type peridotite was left by the differentiation of a continental sialic crust "which had given rise to the pre-drift continental block subsequently split by the opening of the Atlantic rift". It

may be legitimately objected, of course, that the continued existence of such a layer would be unlikely in a region of mantle convection. To this, Bonatti *et al.* bluntly admit that they have no answer. But in support of their hypothesis they point to the apparent lack of peridotite in fracture zones associated with the East Pacific Rise. According to continental drift reconstructions, the Pacific is relatively a much older ocean in which a sialic crust probably never existed and thus in which a residual alpine-type peridotite layer would never have developed. Speculation apart, however, the raw data obtained by Bonatti *et al.* may be taken to support the view that continental alpine massifs are "rinds" of ancient oceanic crust carried to the edge of the continent by seafloor spreading, incorporated into a geosyncline and later uplifted.

Measuring Sea Waves by Radio

A NEW technique to help oceanographers measure sea waves by radio signals is suggested by K. Hasselman in next Monday's *Nature Physical Science* (229, 16; 1971). Previously, only seawaves of a few metres in length could be surveyed satisfactorily by radio signals, but Hasselman has discovered a method applicable to longer waves.

When electromagnetic waves are transmitted nearly horizontally over the sea, the water waves interfere with them to produce backscatter which is characteristic of the water wave spectrum. The salient part of the backscatter is caused by the diffraction grating effect of those wave components with precisely half the length of the electromagnetic waves, travelling in the same or opposite direction. (Such components are always present because the sea wave spectrum is continuous.) The resulting signal has a highly tuned Doppler shift of order 10^{-1} Hz, equal to the frequency of the water waves of said length, which is closely defined by gravity. The strength of the signal, suitably calibrated, is a measure of the sea wave spectrum, which can be scanned by varying the transmitted electromagnetic frequency. These facts, first shown by D. D. Crombie of New Zealand (*Nature*, 175; 681; 1955), have been verified by others, and were used to explain a case of fluctuating television reception near Plymouth. Recently, J. F. Ward (*Nature*, 223, 1325; 1969) has developed a technique for measuring the backscatter from small areas of ocean at distances of thousands of kilometres with gated signals propagated by the ionosphere.

In principle, this sounds like the answer to a wave-oceanographer's prayer—a land based method of continuously surveying the ocean waves, far more convenient and productive than the usual practice of sending men and instruments

to be tossed about by the waves themselves. But the drawback has been that reasonably directional radio beams (which are obviously needed) can be produced only at high frequencies with wavelengths of a few metres, whereas the most important ocean waves have typical lengths of many tens of metres.

Hasselman has now suggested a new way of looking at the backscatter from the longer waves. Besides the highly tuned lines in the Doppler return (and some weaker lines of minor importance) there is also a continuous background spectrum, which shows some structure but whose origin had not yet been explained. Hasselman claims that this background is in fact a fairly direct image of the total sea wave spectrum. His calculations involve second order interaction theory. Although the principal lines may be explained by first order theory in which two waves interact when their wave numbers satisfy certain sum conditions, the second order theory involves triads of wave numbers. The extra degree of freedom causes the whole continuum of sea waves to contribute to the backscatter; for any given sea wave number, a third can be found to balance the sum with the radio wave number. By use of a very reasonable approximation, which appropriately requires the radio wavelength to be short, the spectrum of the backscatter is expressed as the product of an electromagnetic transmission factor, the spectral amplitude of the first order line, the sea wave spectrum, and coupling factor calculable from hydrodynamic theory. The transmission factor can be eliminated by dividing by the first order line, and Hasselman obtains the intriguing result that the ratio of two parts of the same radio signal is a direct measure of the spectral amplitude of sea waves in metres.

Gondwanaland, Palaeomagnetism and Continental Drift

by

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A reconstruction of Gondwanaland linking India, Australia and Antarctica and fitting these against Africa is compared with some geological and geophysical observations and also with the recent reassembly proposed by Smith and Hallam. The probable history of the disintegration and movement of the northern and southern continents into their present configuration is assessed against the available palaeomagnetic data.

Smith and Hallam¹ have recently presented a reconstruction of the southern continents using computer matching of the 500 fathom contours combined with the most plausible fit estimated on geological and geophysical grounds. Using similar techniques but assessing the most plausible fit on different criteria, I have chosen² a radically different reconstruction. Neither of these reconstructions can be considered as the final picture for the period 250–300 million years ago, but they seem to represent the two extremes of the possible assemblages and it should be possible to distinguish between these two basic models.

The techniques used here are similar to but less precise than those of Smith and Hallam¹, Sproll and Dietz³ and Bullard *et al.*⁴. Their reconstructions used precisely mapped contours, sometimes undertaking new surveys for this purpose⁴, which were fed into a computer as a series of closely spaced coordinates. The data were then processed using a "homing" routine which systematically sought the "best fit" between two contour lines. The best fit for this purpose was defined as the minimum amount of overlap and gap. The geometrical reconstruction presented here (Fig. 1) was obtained using a palaeomagnetic computer program which plotted continental outlines in their palaeolatitudinal position relative to a pole determined palaeomagnetically. By the use of arbitrary pole positions, the continental outlines could be superimposed and the best fit determined by eye. For this purpose, the continental edges were taken at the 1,000 fathom contour and irregularly spaced coordinates were selected from *The Times Atlas* so that joining these points gave the approximate shape of the contour. This method is clearly less sophisticated and, to avoid apparent precision, the pole positions used are not given, but it is thought that the results do not differ meaningfully from the more precise methods used by others because there is an inherent lack of precision in determining (a) which contours should be matched, for example, 500 or 1,000 fathoms, and (b) selecting a best fit which excludes certain overlaps—overlaps should not occur except where the contour can be demonstrated to have been altered by sediments or igneous activity subsequent to the separation of the continents.

Smith and Hallam place both India and Australia against Antarctica, and move this against Africa, fitting Madagascar and Ceylon into the gaps. The reconstruction presented here

fits India to Australia and Australia to Antarctica; this is then fitted against Africa without moving Madagascar, but leaving a large triangular gap just north of the Mozambique Channel. The fit of India to Australia was found to be "geometrically acceptable" by Smith and Hallam and the fit of Australia to Antarctica seems to be widely acceptable. The reconstruction chosen for the period 250 to 300 million years ago depends on the available geological and geophysical evidence.

Geology and Geophysics

It is difficult, if not impossible, to evaluate the matching of east India and Antarctica as the outcrop of rocks along the edge of Antarctica is small and has been inadequately studied, but there does seem to be matching geological features between eastern India and north-western Australia; Crawford⁵ has presented preliminary studies of Pre-Cambrian rocks and Ahmad⁶ has outlined some of the Phanerozoic similarities, but the two reconstructions can most easily be tested by examining the evidence for or against the Tertiary movement of Madagascar. All geological studies⁷ in the areas of East Africa and Madagascar bordering the Mozambique Channel have shown it to be "geosynclinal" from at least early Karroo times (late Carboniferous) and it now contains an estimated thickness of 14 km of Karroo and post-Karoo sediments which were laid down in an actively sinking area. Sporadic marine incursions occurred although most sediments exposed on the continents were laid down subaerially. These estimates of thickness, based on sediments mapped on the land surfaces,



Fig. 1 The proposed reconstruction of Gondwanaland based on geometrical fitting with some support from geological and geophysical evidence. The projection is stereographic but no palaeolatitudes are implied by the centre of the projection which was chosen arbitrarily. It is suggested that the continents, with possible exceptions of parts of Asia, retained this configuration for at least a part of the Upper Palaeozoic and early Mesozoic.

have been confirmed to some extent by drilling⁸ and gravity observations⁹ which further suggest a non-oceanic basement to these sediments. More recent geological studies in the Comores Archipelago, midway but slightly north of the Channel, showed "unequivocal evidence that this part of the Indian Ocean is underlain by non-oceanic crust"¹⁰.

Geological investigations on neighbouring coastlines or islands suggest that the "geosynclinal" conditions of the Mozambique Channel extend much farther north^{7,10,14,15} although the sedimentary thickness tends to thin slightly northwards and more rapidly eastwards. Detailed studies on the Kenyan coast¹⁵ suggest that these sediments extend well beyond the 1,000 fathom contour (so that this contour line is inadequate for defining the continental margin). Vertical faulting on the African mainland exceeds 7 km¹⁵ and clearly the Somali Basin to the east has subsided by similar amounts to that observed in the Mozambique Channel. Seismic and gravitational observations in the Somali Basin, between Mombasa and the Seychelles^{16,17}, do not show "normal" oceanic crust except in very restricted areas (where there are also unexplained discrepancies between the seismic and gravitational observations) and confirm the presence of thick sedimentary deposits. This crust seems to be more similar to the "continental" ridges of the South-west Pacific¹⁸ than to newly formed oceanic crust, although the crust is not truly continental. In view of these anomalies and the evidence for persistent subsidence and sedimentation, I consider that the Mozambique Channel has not been created by seafloor spreading during the past 300 million years (with the conjectural exception of small areas near the Amirantes Islands) and therefore Madagascar could not fit into this region during the Mesozoic.

Africa and Microcontinents

On my reconstruction, the eastern boundary of this East African subsiding zone could be represented by the Darling fault region of Western Australia which has had a similar subsiding history since the Lower Palaeozoic. Within this wide subsiding trough, some areas may have been continually marine, and there have been small pockets of true oceanic crust, as in the present day Mediterranean¹⁹ or East Indies²⁰; the Amirantes Islands may even represent a fossil island arc associated with a minor proto-Indian Ocean. Such speculations are premature although they do offer a possible synthesis of various observations.

The positioning and extent of the "microcontinents" of the Indian Ocean—the Seychelles^{21,22} and possibly the Chagos-Laccadive, Ninetyeast, Mascarene, Madagascar and Mozambique Ridges²³—are somewhat uncertain. It is possible that some extra closure should be made if some of these ridges are not continental, but this would require only a small modification to my reconstruction.

The most serious geological disadvantage of this reconstruction is the relationship which results between Antarctica and South Africa–South America. This can be improved if it is assumed that there has been some differential movement between East and West Antarctica; some such movement is implied on most reconstructions.

For the northern continents, I suggest one small modification to the Bullard *et al.*⁴ reconstruction. Europe and Greenland have been moved slightly northwards relative to North America. This minor modification is within the accuracy of the definition of the continental edge within the Labrador Sea and has the advantage that the area between north-western Greenland and Ellesmere Island behaves as a simple transcurent fault and not a compression zone, and the Mediterranean is opened slightly between Spain and Morocco (previously⁴ an overlap) allowing the accumulation of the sediments forming the Betic and Rif nappes before their late Tertiary deformation.

Palaeomagnetic Reconstructions

The magnetization of the ocean floor has been measured for many decades although the significance of these measurements has only been realized since the concept of seafloor spreading²⁴. The analysis of these earlier records and completion of new

traverses will eventually lead to the detailed picture of the way in which the continents separated, but in the meantime it is necessary to examine the evidence already available from palaeomagnetic studies undertaken on continental rocks²⁵. These data are of variable quantity and quality and must be selected carefully using the reliability of its palaeomagnetic and age characteristics. The data used (Table 1) in the reconstructions (Figs. 2 and 3) have been selected as far as possible on the basis of laboratory evidence for stable remanence for undisturbed rocks, but it is impossible to use proper stringent criteria because there is still little fully reliable evidence. This means that most latitudinal positions can only be regarded as accurate within some 5° in the more reliable cases, whereas the longitudinal positions for the Mesozoic–Tertiary have been determined subjectively, assuming as small a longitudinal change as practicable.

(a) *Mesozoic–Tertiary*. The agreement of Africa and South American pole positions is quite good throughout this period if some rotational opening of the South Atlantic is permitted in the late Cretaceous. The evidence from India is not very consistent with either reconstruction and there are even fewer possibly reliable data for Antarctica. The Jurassic position of Antarctica is, however, more consistent with the Smith and Hallam model, although the evidence for it may be incorrect because it is inconsistent with the Jurassic position of Australia which is based on much better data.

The data for the Australian Mesozoic–Tertiary are of good quality and clearly place Australia much farther south than would be expected on the Smith and Hallam reconstruction, but are consistent with this model and supported by the palaeoclimatic evidence^{26,27}. The chief discrepancy with the proposed model is that with Australia fixed, this places Antarctica slightly to the south of Africa, yet there seem to be indications of a land mass off south-eastern Africa in at least late Carboniferous–Permian times²⁸ until, possibly, the Cretaceous¹². The position of most southern continents is particularly well fixed between 100 and 60 million years ago and is also more consistent with the evolution of the proposed model than with the evolution of the Smith and Hallam reconstruction.

(b) *Palaeozoic*. There is less palaeomagnetic information available for the Palaeozoic and it is generally less reliable. There is still quite good agreement between the South American and African polar wandering curves and these curves are supported by palaeoclimatic evidence of ice caps in the Sahara during the Ordovician–Silurian^{29,30} and in Southern Africa and Brazil³¹ during the Carboniferous–Lower Permian.

The Indian data so far available are variable; the individual pole determinations for the Carboniferous and Permian cannot be averaged in any meaningful way but are, in any case, little different on either reconstruction.

The Antarctic Ordovician results (D1) are in good agreement with each other and, on the Smith and Hallam reconstruction, they coincide with the Ordovician palaeoclimatic evidence of polar conditions in the Sahara. Unfortunately, the mutual agreement of the palaeomagnetic data indicates that it is associated with a later remagnetization, for this agreement is lost if correction is made for subsequent tectonic disturbances³².

The Australian Palaeozoic polar wandering curve is based on four apparently reliable observations (C1–C4). The new Cambrian result (C1)³³ lies close to the previously accepted Cambrian position on the Smith and Hallam reconstruction. Unfortunately it is difficult to assess where the true Cambrian pole lies at this time; there are now three radically different pole determinations from African Cambrian rocks (B1) and the precision of the South American determination (A1) is extremely poor. Palaeoclimatic considerations have suggested some cooling in the Cambrian of Morocco³⁴, but these purple rocks are identical, except in colour, to red currant bedded desert sandstones above and below them, and the presence of limestones further supports a generally low to mid-latitudinal location at that time, suggesting that the pole is at least some 40°–50° away from Morocco at this time. Current studies may soon clarify the Cambrian situation, but at the moment the

Table 1 Phanerozoic Palaeomagnetic Data used in Figs. 2 and 3.**(a) Mesozoic-early Tertiary palaeomagnetic data**

Pole positions			
	N	E	Reference
Palaeocene and Eocene			
Europe	77	161	41
Greenland	63	174	42
N. America	66	239	43
Africa	81	168	44
India	33.5	281.5	45
Australia†	60.5	318.5	46, 47
Antarctica	86	178	48
† Data may include Oligocene			
Cretaceous			
N. America	68.5	178	43, 49–53
S. America	78	234	59
Africa	61	261	54, 55
India	30	287	56
Australia	53.5	328.5	57, 58
Jurassic			
N. America	54	186	52, 60
S. America	84	236	61
Africa	64.5	265.5	62–66
India	14	297	67, 68
Australia	44.5	324	58, 69, 70
Antarctica	54	39.5	72–74
Triassic			
Europe	48	152	41
Asia	40	203	41
N. America	65	104.5	75–83
Africa	76	231	84, 85
S. America	83	22.5	86
Australia	53.5	332.5	58, 70

* This differs from the pole usually quoted (84S 46E). Unfortunately the pole position calculated from the direction before and after correction for tectonic tilt had been transposed in the original article (see text) and this slip has been incorporated in most subsequent reviews.

† Small errors have been reported³² on the original pole determinations and the corrected values are given here.

(b) Palaeozoic Gondwanan data

Africa	Age	Formation	Pole position		Ref.
B 1'	Cambrian	Morocco (Helsley)	53N	34E	100
B 1''	Cambrian	Morocco (Hailwood)	31N	105W	—
B 1'''	Cambrian	Ntonya Ring Structure	28N	15W	87
B 2	Ordovician	Hook Intrusives	14N	26W	88
B 3	Devonian	Red beds	17N	40E	—
B 4	Carboniferous	Dwyka	26S	26E	89
B 5	Carbo-Permian	K3, Galula	45S	40E	85
B 6	Permian	K3, Songwe	27S	89E	85
South America					
A 1	Cambrian	Red beds	27N	50W	59
A 2	Silurian	Uruncum	34N	16W	90
A 3	Devonian	Red beds	13N†	19W†	90
A 4	U. Carboniferous	Taiguati	28S	34W	90
A 5	U. Carboniferous	Piaiu	65S†	10W†	90
A 6	Carbo-Permian	Paganzo	60S	6W	37
A 7	Carbo-Permian	Paganzo	57S	22W	37
A 8	Permian	Red beds	66S†	34W†	90
A 9	Permo-Triassic	—	77S	175E	86
A 10	Permo-Triassic	—	78S	141W	86
Antarctica					
D 1'	Ordovician	Lutzow-Holm	19S	23E	98
D 1''	Ordovician	Sør Randane	29S	10E	32
India					
E 1	Cambrian	—	28S	32E	33
E 2	U. Carboniferous	Talchir	32S	46W	99
E 2''	U. Carboniferous	—	4N	77W	97
E 3'	Permian	Kamthi	19S	128E	92
E 3''	Permian	Kamthi	21S	50W	97
Australia					
C 1	Cambrian	—	9S	20W	33
C 2'	Silurian	Mugga Mugga-thermal	80S†	20W†	93
C 2''	Silurian	Mugga Mugga—a.c.	69S	40E	93
C 3	M. Carboniferous	Lower Kutung	67S*	110W*	36
C 4	U. Carboniferous	Paterson Toscanite	73S	147E	36
C 5	U. Carboniferous	Main Glacial	53S	148E	36
C 6	U. Carboniferous	Curabubulla	43S	135E	36
C 7	U. Carboniferous	Rocky Creek	52S	138E	36
C 8	U. Carboniferous	Percy Creek	(23S	138E)	94
C 9	Permian	Lower Marine	(46S	122E)	95
C 10	Permian	Upper Marine	44S	132E	95
C 11	Permian (?)	Moonbi	(35S	128E)	36
C 12	Permian	Milton Monzonite	32S	170E	96

evidence is inadequate to test any Gondwanan reconstruction for this period.

The Australian Mugga Mugga Porphyry of Silurian-Devonian age (C2) has two possible poles; both are at least 40° away from the palaeoclimatic pole in the Sahara on either reconstruction. This pole position appears equally inconsistent with either reconstruction. The Australian Lower Kutung pole position (C3), Viséan³⁵, is slightly more consistent with the Smith and Hallam reconstruction, but there is considerable uncertainty about the tectonic correction³⁶, although it seems likely that corrections would improve the agreement with the Smith and Hallam model.

In summary therefore the available Palaeozoic data seem to be slightly more in agreement with the Smith and Hallam reconstruction. There are grounds for considering that at least some of the present data are not, in fact, sufficiently precise for testing different reconstructions for this period of time or for evaluating the postulated³⁷ Permian drifting of Australia away from the rest of Gondwanaland.

Palaeomagnetism and Drifting

In the late Palaeozoic, the continents seem to have had a configuration about the Atlantic which is almost identical to the geometrical reconstruction of Bullard *et al.*⁴ and the evidence for the configuration around the Indian Ocean has just been outlined. Pangaea, in the form of a U-shaped continent, existed by late Palaeozoic times, with the intervening Tethys Ocean occasionally transgressing over the continents to form two land masses at certain times. This general configuration could have persisted from the start of the Phanerozoic³⁸ although the presence of some orogenic periods has been used to suggest^{39,40} major movements earlier in this period.

In the Triassic, the geological evidence suggests that the

continents maintained this general relationship, but there is a very distinct discrepancy in the palaeomagnetically determined position of Europe relative to North America and Africa. Work in progress on Moroccan rocks confirms the African data, and the agreement of this with North and South American data suggests that the European data, which are entirely on undemagnetized material⁴¹, are systematically in error. A pilot demagnetization study of some British Triassic rocks¹⁰¹ seems to confirm the European pole position but detailed studies are required to solve this discrepancy.

A further discrepancy occurs between the palaeomagnetic determinations of the European Permo-Triassic poles which lie in or near the Eastern Maritime Provinces of the Soviet Union, yet the palaeoclimatic factors suggest tropical, warm water environments¹⁰² in that region. If the Eurasian block was not then behaving as a single rigid block, these parts of Asia could then have lain farther south, becoming welded onto the Eurasian land mass later, possibly in association with the eruption of the Triassic Siberian Traps. The only drifting indicated in the western hemisphere was a slight opening by a few hundred kilometres, possibly 300 km, of the proto-Central Atlantic, between North Africa and Eastern North America. This trough accumulated the sediments which now lie on the edge of the North American continental shelf¹⁰³, and its opening was possibly associated with Triassic eruptions in eastern North America and North Africa.

Eastern Gondwanaland seems to have begun to split up in the mid Jurassic^{11–13} when marine incursions spread along the east coasts of India and Africa, generally following earlier structural patterns. The palaeomagnetic data suggest some separation of India from Australia at this time but Antarctica can be presumed to be still connected to Australia because this position is still indicated as late as the Palaeocene. Antarctica,

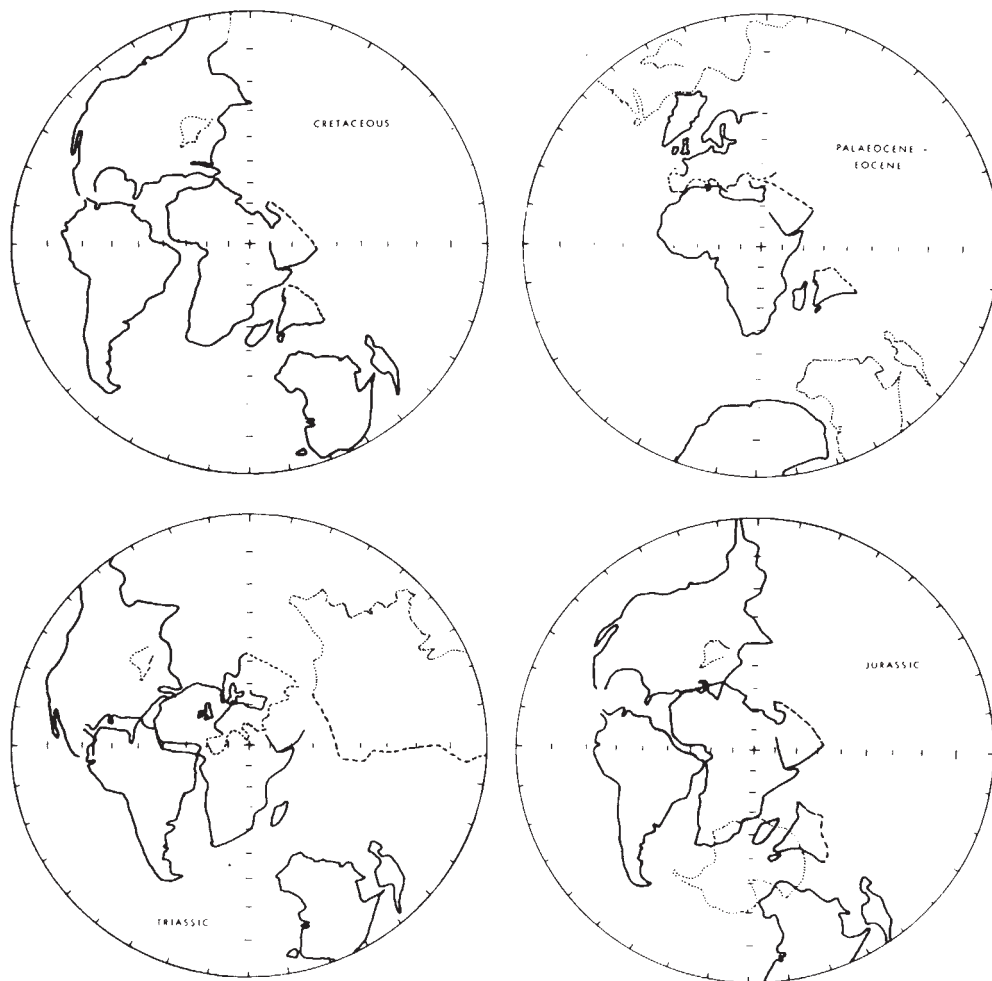


Fig. 2 The palaeolatitudinal relationships of the continents in the Mesozoic-early Tertiary. The more reliable palaeomagnetic data (Table 1a) have been used to determine the latitude and orientation relative to the pole positions, but the longitudes have been determined subjectively, although keeping longitude changes as small as is feasible.

in fact, seems to have remained in polar latitudes from Permian times to the present day. The proto-Indian Ocean does not seem to have passed around South Africa until late Cretaceous¹², yet the palaeomagnetic evidence suggests some earlier separation between Antarctica and South Africa. This discrepancy may reflect errors in the palaeomagnetic determinations although they are consistent throughout the Mesozoic data. The details of the spread of these marginal seas around Africa and South America as the South Atlantic formed have recently been documented¹⁰⁴; continuous marine connexion eventually taking place in the Lower Turonian (approximately 92 million years ago). The palaeomagnetic data suggest that this initial opening took place as a rotational movement of South America away from Africa, before an east-west translation, but the palaeomagnetic data show a persistent slight opening of South America compared with Africa throughout this time.

In the North Atlantic, European palaeomagnetic evidence is lacking for the Jurassic and Cretaceous, and it is difficult to estimate the opening of the Labrador Sea and Bay of Biscay. The presence of mid Jurassic dykes in West Greenland^{105,106} which parallel the Labrador Sea may indicate that this was opening at this time, and there is evidence¹⁰⁷ that, in the Cretaceous, marine conditions spread along it from the north towards the south. There is good palaeomagnetic evidence⁴² that the North Atlantic did not exist between Greenland and Europe in Eocene times, but began to form immediately after this period.

The Smith and Hallam reconstruction¹ cannot be considered unique from morphological considerations alone and other

fits of Africa, India, Australia and Antarctica are possible; the most likely fit for a period some 300 million years ago must be tested rigorously using other criteria. There is some geological evidence of matching features between some continents, but the key to the most appropriate reconstruction for this period seems to be centred around Madagascar. If Madagascar has not moved significantly relative to Africa during the past 300 million years or so, then this effectively locks the pattern. Although the present evidence cannot be regarded as conclusive, it seems probable that Madagascar has not moved away from the Kenyan coast during this time. The suggested pattern seems to be confirmed by palaeomagnetic observations on Mesozoic and Tertiary rocks from the Gondwanan continents, but it is difficult to evaluate the significance of available Palaeozoic results.

Assuming a reconstruction similar to that presented here, a coherent picture can be made of the way in which the continental distribution has evolved into its present pattern. This evolution indicates the general agreement between the palaeomagnetic, geological and palaeoclimatic evidence is very good. The only apparent discrepancy with the plate tectonic concepts lies in the north-east Indian Ocean. This area is aseismic⁹¹ and is considered as a part of the Indo-Australian rigid plate⁷¹, yet the palaeomagnetic evidence indicates a rotation of these two continents relative to each other (irrespective of the initial reconstruction adopted). This rotation may have ceased as India impinged against Asia possibly some 10 million years ago when there also seems to be evidence of slight changes in the direction and rate of seafloor spreading and major tectonic episodes throughout the Alpine-Himalayan and circum Pacific

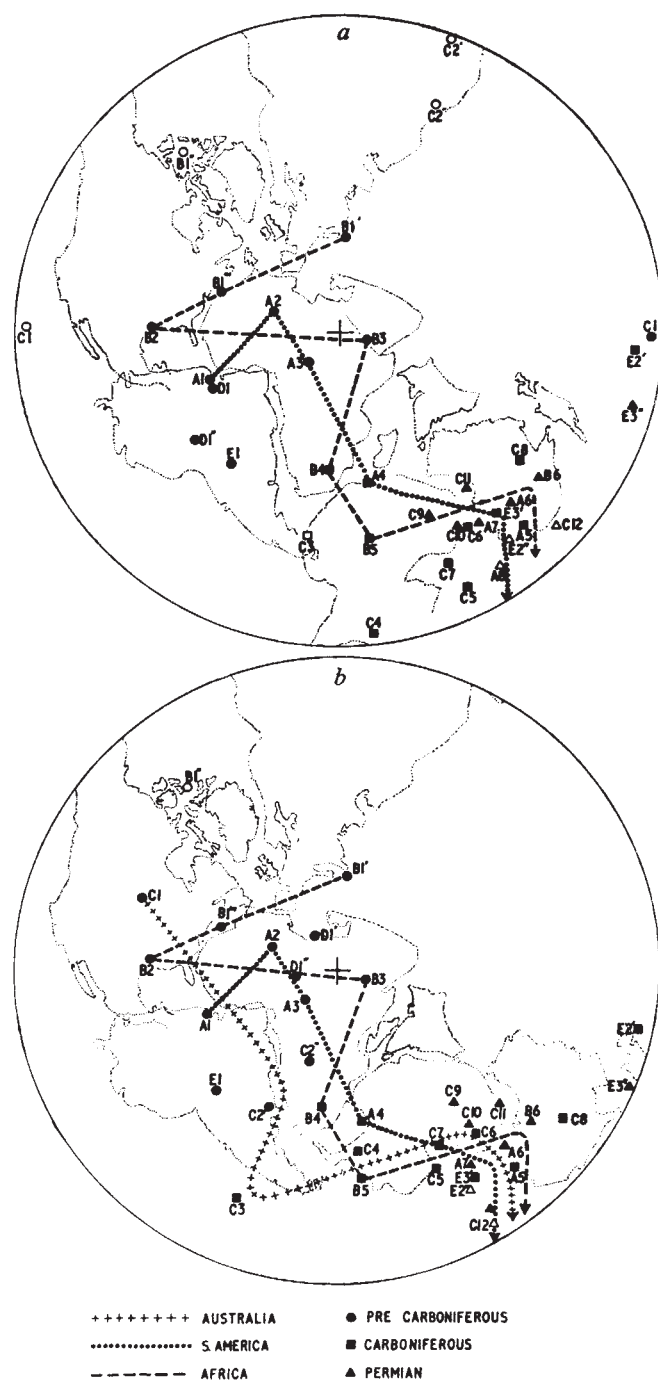


Fig. 3 The Palaeozoic palaeomagnetic poles and postulated paths in the Palaeozoic. *a*, Plotted for the continental outline of Fig. 1; *b*, plotted for the Smith and Hallam¹ reconstruction. The projections are stereographic. The pole data are given in Table 1b.

regions. On the whole, however, the agreement between the different techniques gives a remarkably consistent picture during the past 300 million years of the evolution of the continental distribution.

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Essential Chemical Requirements for Induction of Allergic Encephalomyelitis

by

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Several peptides related to the encephalitogenic tryptic peptide, Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys(Arg), which is derived from the A1 protein, have been synthesized and tested for induction of experimental allergic encephalomyelitis. The Trp - - - - Gln-Lys(Arg) relationship seems to be essential.

EXPERIMENTAL allergic encephalomyelitis (EAE) is an experimentally produced inflammatory and demyelinating disease of the central nervous system (CNS)^{1,2} that occurs when the animal becomes sensitized to CNS tissue or to the basic protein from CNS myelin³⁻⁵. It seems to be mediated primarily by cells associated with delayed hypersensitivity rather than circulating antibody². As an animal model for autoimmune disease, EAE has been the subject of investigation for many years⁶⁻⁸; it seems to be similar to some human demyelinating diseases such as multiple sclerosis both on a clinical and histological basis⁹. The basic protein (A1 protein) constitutes 30% of the total myelin protein. It has a molecular weight of nearly 18,000 and numerous experiments have demonstrated an open conformation¹⁰⁻¹³.

Chemical definition of the encephalitogenic determinants was made possible by the finding that from both the peptic¹³ and tryptic¹⁴ digests of the bovine A1 protein, relatively small peptides of fourteen (E peptide) and nine (T27 peptide) residues respectively could be isolated with high encephalitogenic activity. These peptides are derived from the same unique region of the polypeptide chain that surrounds the single tryptophan residue^{14,15}. The peptides and the intact protein have approximately the same encephalitogenic activity on a molar basis.

We have extended this study by sequencing the amino-acids of the encephalitogenic peptides^{14,15} and by chemically synthesizing an encephalitogenic peptide of eleven residues¹⁴.

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The synthetic peptide, the sequence of which matched the derived peptides, induced clinical signs (hind leg paralysis, weight loss and tremors) and histological lesions (perivascular cuffing and so on) characteristic of EAE and identical to those induced by the A1 protein itself and the derived peptides¹⁴. The object of the work reported here was to determine which of the amino-acid residue side chains are essential for EAE induction. Using Merrifield solid phase peptide synthesis technique^{14,16,17}, we synthesized several analogous peptides, each differing from the natural peptide by one residue. A comparison of the encephalitogenic activities permits a more precise definition of the disease inducing site.

Peptide	Encephalitogenic	Sequence
T 27	+	Phe Ser Trp Gly Ala Glu Gly Gln Lys
E	+	Ser Arg Phe Ser Trp Gly Ala Glu Gly Gln Lys Pro Gly Phe
S1	+	Ser Arg Phe Ser Trp Gly Ala Glu Gly Gln Lys
S2	-	Ser Arg Phe Ser Trp Gly Ala Glu Gly Gln
S3	+	Ser Arg Phe Ser Trp Gly Ala Glu Gly Gln [Arg]
S4	-	Ser Arg Phe Ser Trp Gly Ala Glu Gly Gln [Ile]
S5	-	Ser Arg Phe Ser Trp Gly Ala Glu Gly [Ile] Lys
S6	+	Ser Arg Phe Ser Trp Gly Ala [Ile] Gly Gln Lys
S7	-	Ser Arg Phe Ser [Phe] Gly Ala Glu Gly Gln Lys
S8	-	Ser Arg Phe Ser [Val] Gly Ala Glu Gly Gln Lys
S9	+	Ser Arg Phe [Ala] Trp Gly Ala Glu Gly Gln Lys
S10	+	Ser Arg [Val] Ser Trp Gly Ala Glu Gly Gln Lys

Required Sequence - - - - Trp - - - - - Gln Lys

Fig. 1 The sequence of the synthetic peptides used in this study are shown along with tryptic peptide T27 and peptide E.

The peptides used in this study (Fig. 1) include tryptic peptide T27 and peptic peptide E which were derived from the bovine A1 protein, purified and sequenced as described earlier^{13,14}. The synthetic peptides, S1-S10, were made by Merrifield solid phase peptide synthesis¹⁴. After removal from the resin the peptide was further purified by gel filtration in 0.5 M acetic acid using a layered column of 'Sephadex

G-10' and 'G-25' (4×100 cm). In each case, the elution pattern, determined by absorbance at 280 nm, contained a single peak which eluted before various lesser quantities of smaller peptides. High voltage electrophoresis at pH 4.6 showed that this peak was homogeneous; the trailing material often contained two to four peptides which migrated differently from the major peptide. Based on the relative areas of the major and minor peptide fractions, the yield varied from 60–90%. Approximately 200–400 mg of purified peptide was usually obtained. The amino-acid sequences of some of the synthetic peptides, determined by Beckman amino-acid analyser¹⁴, are shown in Table 1. The amino-acid residues were present in the peptide hydrolysates in integral molar ratios within $\pm 10\%$, the limits of the amino-acid analyser.

Synthetic Peptides

The peptides shown in Fig. 1 and Table 1 were selected for synthesis because they differed from the native sequence by only one amino-acid. Because the length limit was previously established¹⁴ as nine residues by the tryptic peptide T27, the N-terminal Ser-Arg region of peptide E is clearly not essential for encephalitogenic activity. The peptides had been synthesized, however, before this was known. Within the nine residue sequence, the phenylalanine, serine, tryptophan, glutamic, glutamine and lysine residues were selectively replaced; the two glycine and single alanine residues were retained in all cases.

Table 1 Amino-acid Analysis of Synthetic Peptides

Peptide*	Ser†	Arg	Phe	Trp‡	Gly	Ala	Glu	Lys	Val	Ile
S1	1.95	0.89	0.99	—	1.99	1.00	1.85	1.05	—	—
S2	1.88	0.85	0.94	—	1.98	1.00	1.87	—	—	—
S3	1.95	1.87	0.95	—	2.00	1.00	1.95	—	—	—
S4	1.99	0.85	0.97	—	1.87	1.00	1.87	—	—	0.89
S5	2.01	1.01	0.89	—	2.04	1.00	1.10	1.15	—	1.00
S6	1.87	0.95	1.01	—	2.05	1.00	1.01	1.14	—	0.87
S7	1.94	0.89	2.03	—	2.11	1.00	1.83	1.11	—	—
S8	1.88	1.00	0.87	—	2.03	1.00	2.00	1.05	1.03	—
S9	2.00	0.87	0.93	—	2.07	2.00	1.91	1.13	—	—
S10	1.94	0.95	—	—	1.95	1.00	1.90	1.10	0.89	—

* Based on 1.00 for alanine.

† Corrected for degradation of serine during acid hydrolysis (approximately 25%) performed at 110°, 24 h.

‡ The tryptophan value, evaluated by absorbance analysis, was generally near 1 mol/mol peptide.

Table 2 and Fig. 1 summarize our results. The activities fall in two categories: peptides are either highly encephalitogenic or inactive. Thus, on the basis of the EAE assay, a distinction can be made between the replaceable and non-replaceable amino-acid residues. We see that the specific side chains of tryptophan, lysine and glutamine are required for activity. In addition, the relative positions of these three residues seem to be determined by the four internal residues, the functional groups of which may not be essential.

The encephalitogenic activities of the synthetic peptides, given in complete Freund's adjuvant, are shown in Table 2. No evidence of clinical or histological signs of EAE were found in any of the animals tested with peptides S7 and S8 where phenylalanine and valine were substituted for the tryptophan, thus revealing an essential role for this residue. The lysine and glutamine residues are also essential; deletion of the lysine (S2) or replacement by isoleucine (S4) produces inactivity. In one animal out of twelve tested with the isoleucine replacement for glutamine (S5) a few minor lesions were found using 3.3 μ g of peptide. The other residues which might possibly influence the activity, the glutamic, serine and phenylalanine residues, are relatively unimportant. In comparison with peptide S1, active at 0.33 μ g, the smallest concentration tested, the requirement for the tryptophan, gluta-

mine and lysine residue side chains is at least one hundred times that of any other residue side chains.

Significance of the Determinant

At least three residues (tryptophan, glutamine and lysine) seem mandatory for disease induction. It can be concluded, therefore, that EAE induction in guinea-pigs is primarily related to a unique linearly spaced array of these three residues — Trp — — — Gln, Lys (Arg).

We found that arginine would substitute fully for lysine. The requirement, therefore, at the sixth position from the tryptophan residue is not exclusively lysine but rather a positively charged side chain. This is in accord with the comparative encephalitogenic activities of the human and bovine A1 proteins which are indistinguishable on the basis of encephalitogenic assay in guinea-pigs¹¹. The human protein contains arginine^{20,21}, whereas lysine is found at that position in the bovine A1 position¹⁵.

Table 2 Encephalitogenic Activity of Peptides

Peptide	Dose (μ g)	Encephalitogenic activity*	
		Clinical	Clinical + histological
S1	0.33	1/4	3/4
	3.3	2/4	4/4
	33	3/4	4/4
S2	3.3	0/4	0/4
	33	0/4	0/4
	33	2/4	3/4
S3	10	2/4	3/4
	33	3/4	4/4
	33	0/4	0/4
S4	10	0/4	1/4
	33	0/4	1/4
	33	0/4	0/4
S5	3.3	0/4	1/4
	10	0/4	0/4
	33	0/4	0/4
S6	3.3	0/4	4/4
	10	3/4	3/4
	33	2/4	3/4
S7	3.3	0/4	0/4
	10	0/4	0/4
	33	0/4	0/4
S8	3.3	0/4	0/4
	10	0/4	0/4
	33	0/4	0/4
S9	3.3	2/4	2/4
	10	2/4	3/4
	33	2/4	3/4
S10	3.3	1/4	2/4
	10	3/4	3/4
	33	3/4	3/4

* Encephalitogenic activity is expressed as the number of guinea-pigs showing clinical and/or histological signs of EAE over the total tested as described earlier³. Onset was generally at 10–11 days after injection although in some cases the clinical signs were not observed until the nineteenth or twentieth day. All animals were killed on the twentieth day for histological examination. In these test conditions, the bovine A1 protein (20 μ g) showed ten out of ten animals with clinical signs of EAE with the onset 10–11 days after intradermal injection in complete Freund's adjuvant (Difco, *M. tuberculosis*) in the hind footpads.

The space-filled model of peptide S1 is shown in Fig. 2. It is interesting that the proximity of the phenylalanine, a non-essential residue, to the tryptophan could provide a degree of π -orbital interaction. This type of interaction is apparently of minor importance because valine can substitute for phenylalanine (peptide S10). It was previously suspected, however, that the phenylalanine might be an essential residue¹², for a peptic peptide beginning with NH_2 -Ser-Trp-Gly- was relatively inactive¹¹. A reasonable explanation is that the close proximity of the N-terminal amino group to the tryptophan is inhibitory. In terms of chemical structure, therefore, at least three side chains seem to be critical, the foremost being the tryptophan ring structure. We are investigating

whether the internal glycine and alanine residues are required for activity.

Our data reveal three facts which suggest that the tryptophan region of the A1 protein is the major encephalitogenic site; (a) the derived and synthetic peptides have approximately equal activity to the A1 protein on a molar basis; (b) the HNB-A1 protein^{14,15}, in which the tryptophan residue is exclusively modified by reaction with 2-hydroxy-5-nitrobenzyl bromide, is non-encephalitogenic at concentrations a hundred times that of the A1 protein; (c) of A1 proteins from several mammalian species, including man, monkey, dog, rabbit, guinea-pig, rat, mouse and horse, all of which are equally encephalitogenic in guinea-pigs, the specificity of the Trp-Gln-Lys (Arg) is preserved²¹. Only in the chicken and turtle A1 proteins²¹, which are non-encephalitogenic, is this sequence changed. Thus it seems that the chemical basis for disease induction in guinea-pigs is primarily accounted for by the tryptophan region. There is, however, an encephalitogenic peptide²² of forty-three residues that is derived from another portion of the A1 protein and which is encephalitogenic in rabbits. But this peptide is non-encephalitogenic in guinea-pigs²⁰ and it seems likely that the region(s) of the A1 protein responsible for EAE induction may vary from species to species.

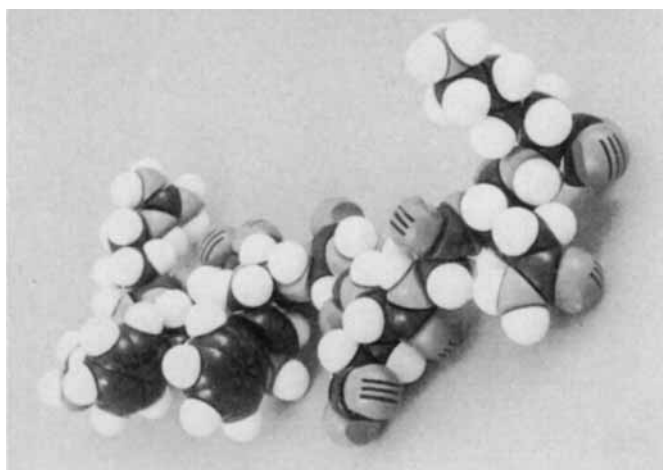


Fig. 2 A possible conformation for synthetic peptide S1 is shown by the space filled model.

The work described here bears directly on the understanding of the size and specificity of the antibody combining site functional in delayed hypersensitive systems. Little information²³ is available about the immunogenic peptides which are capable of inducing an immunogenic response involving sensitized cells or circulating antibody. Most of the data on the size and specificity of the antigenic determinants comes from study of synthetic polypeptides²⁴ and the isolation of peptides which combine with antibody from proteins of known sequence. It is likely that the antigenic determinants of proteins are mainly composed of tertiary sites rather than primary structure because, unlike the A1 protein, most proteins exist in a globular conformation. Thus the encephalitogenic peptide is unusual in that it itself induces the immunopathologic response leading to EAE.

The antigenic determinants of proteins seem to be composed of a series of amino-acids, each of which makes a specific contribution to the binding energy²³. Our data offer striking evidence for this concept with regard to the three essential residues. In this case the binding seems to be of the "all or none" variety: all three residues are required. In the case of tobacco mosaic virus, however, a peptide region of only five residues has been found to contain an antigenic determinant²⁵. This peptide has now been synthesized and the specificity

determined²⁶. It is interesting that, although this peptide is not immunogenic, it makes positive responses characteristic of the delayed hypersensitive reaction in animals previously injected with the virus²⁷. This peptide therefore differs from that reported here in that peptide S1, while inactive in the delayed skin test, is immunogenic¹⁵. The ability of peptides to induce disease but not the delayed-skin reaction could be explained in several ways, foremost of which is the absence of carrier in the test system²⁸. In this sense, the assay for disease is more sensitive than the skin test.

With respect to the dimensions of the antibody combining site, our results are compatible with data obtained with synthetic polypeptides²³. The distance between the tryptophan and lysine residues is approximately 30 Å if the distance between side chains is assumed to be 5 Å. Studies with poly-alanine and polylysine²⁸ have shown that a penta or hexapeptide represents the maximum combining site. Heptapeptides have been derived from myoglobin which combine with antibody to apomyoglobin³⁰. Thus the size of the combining site for delayed hypersensitive reactions as found for the tryptophan region is approximately equal to that involving antibody.

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Properties, Structure and Possible Neuroreceptor Role of the Encephalitogenic Protein of Human Brain

by

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The principal determinant region of the encephalitogenic protein, the amino-acids of which have been sequenced, is located round the sole tryptophan residue. The determinant may be the receptor site for 5-hydroxytryptamine in certain regions of the central nervous system, and the paralysis in experimental autoimmune encephalomyelitis may be caused by an immunopharmacological block of nervous transmission.

MYELIN from the central nervous system contains 23% protein, approximately 30% of which is unusual, with a high content of basic amino-acids¹. Injection of only 1 µg of this protein in Freund's complete adjuvant induces in guinea-pigs experimental autoimmune encephalomyelitis (EAE) which is characterized by paralysis and infiltration of lymphocytes into the brain and spinal cord². EAE is considered to be a model for the human disease multiple sclerosis³. Since the discovery^{4,5} that peptides from the protein could induce EAE, intensive efforts have been made to identify, isolate and characterize the encephalitogenic determinants⁶⁻⁸. I wish to describe the structure of the encephalitogenic basic protein from human brain and to discuss a possible role in the functioning of parts of the central nervous system (CNS).

Properties of Encephalitogenic Proteins

The isolation and encephalitogenic activity of the basic protein from the CNS of several species have been studied before^{2,9}. No marked differences between the human protein¹⁰ and those from other species have been observed except in the rat, which has a large and a small basic protein¹¹. The physical properties of bovine protein have been determined in several laboratories, and selected results are summarized in Table 1. The basic protein is similar in shape and isoelectric point to histones, and as with histones the molecular weight was difficult to determine¹². But this basic protein differs from histones in its higher content of histidine and proline⁶, and it seems to occur only in the CNS. Basic proteins of quite different amino-acid composition exist in peripheral nervous tissue¹³.

Amino-acid Sequence of Human Protein

The protein used for the determination of the amino-acid sequence was obtained from human brains which were defatted with chloroform-methanol^{9,10,14}. After ion exchange chromatography⁶, the protein seemed to be homogeneous by electrophoresis at pH 2.5 in 4 M urea in a 15% acrylamide gel and it had the same mobility as the bovine protein⁶. The amino-acid sequence of the N-terminal¹⁵ and C-terminal¹⁶ regions and the sequence around the sole tryptophan residue in the protein have been reported before¹⁷.

Table 1 Some Properties of Encephalitogenic Basic Proteins

Bovine protein	Property
Molecular weight	19,500 (gel filtration ¹²) 16,400 (sedimentation equilibrium ⁴⁷) 16,500 (amino-acid composition ⁶)
Overall shape	Prolate ellipsoid, axial ratio 10:1 (viscosity ⁴⁷)
Ultraviolet extinction coefficient	$E_{1\text{ cm}}^{1\%}$ at 276 nm 5.89 (ref. 47)
Helical content	0% (optical rotatory dispersion ⁴⁸)
Human protein	
Molecular weight	18,406 (amino-acid sequence—this article)
Isoelectric point	> 10.5 (this article)

Evidence for the sequence was obtained by analysis of peptides produced by digestion of the protein (200–500 mg) with trypsin, pepsin and thermolysin. The peptides were isolated by gel filtration, paper electrophoresis and paper chromatography¹⁵⁻¹⁷. Large peptides were further digested with chymotrypsin, subtilisin, carboxypeptidase and aminopeptidase O. (Full details on the characterization of the peptides will be reported elsewhere.) The peptides which were used to obtain overlaps for the tryptic peptides are shown in Fig. 1.

The amino-acid sequence of the human CNS protein (Fig. 1) has several interesting features. The N-terminal residue is blocked with an acetyl group¹⁵. Hashim and Eylar¹⁸ showed that the corresponding bovine protein is also blocked by an acetyl group but that there is a deletion of His-Gly at residues 10 and 11. Originally only one methionine residue was found in the human protein¹⁵; later a second was located in peptides from the C-terminal end of the protein^{16,18}.

At residues 44–45 and 88–89 the sequence Phe-Phe occurs. This bond seems to be particularly susceptible to pepsin and

to the acid proteinase present in brain. When the protein is isolated from bovine spinal cord defatted with acetone rather than chloroform-methanol, the acid proteinase¹⁹ causes extensive hydrolysis of the protein during extraction with acid⁶. Kibler *et al.*²⁰ have isolated a highly basic polypeptide of forty-five residues from bovine spinal cord which is closely similar at the N-terminal end to residues 45 to 65 in the human protein, except that it differs markedly at the C-terminal end from residues 66 to 88. They have postulated that the regular spacing of non-polar and polar residues facilitates binding to lipids²⁰. Palmer and Dawson²¹ have shown that the protein readily forms complexes with triphosphoinositide.

Microheterogeneity in the Protein

The encephalitogenic basic protein from several species, including man, although it seems homogeneous at an acid or neutral pH, has an unexplained heterogeneity when subjected to electrophoresis at pH 10.6 (ref. 22). This heterogeneity in human protein can be explained by the occurrence of one of a pair of unusual basic amino-acids at residue 107. These amino-

acids were eluted between histidine and arginine from a 'Technicon C-2' resin. From a comparison with the chromatographic properties of methyl- ω -N-guanidino-arginine²³ one is probably monomethyl-arginine and the other dimethyl-arginine. Further work on the isolation and characterization of these amino-acids is in progress. It is noteworthy that Paik and Kim²⁴ found that the activity of an enzyme which methylates arginine in proteins was higher in brain than in other tissues in the rat.

Encephalitogenic Determinants

Methylated arginine occurs close to the principal encephalitogenic determinant in the protein, which is composed of residues 111 to 121. Peptic peptides of fifteen and twenty-two amino-acids from this region (residues 111 to 125 and 111 to 132) were almost as encephalitogenic, on a molar basis, as the parent protein²⁵. This determinant was first isolated⁷ from the bovine protein. Two other regions of the human protein can also induce EAE. They are much less potent, however, when compared on a molar basis; the N-terminal region (residues 1 to 21) is approximately 1,000 times less active and the region from residue 45 to 88 is 500 times less active than the region from 111 to 132 (ref. 25).

The peptic peptide of fifteen amino-acids which contains the principal encephalitogenic determinant has the sequence Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Arg-Pro-Gly-Phe-Gly and is a potent antigen, for as little as 180 pmol in adjuvant induce paralysis in guinea-pigs and delayed hypersensitivity²⁵ to the parent protein. Not all the fifteen amino-acids are essential for the induction of EAE. An examination of peptides synthesized and tested²⁶ shows that Pro-Gly-Phe-Gly from the C-terminal end is not required for activity. A synthetic peptide (supplied by Dr Eylar) comprising the first eleven residues in the peptic peptide produced severe EAE in guinea-pigs²⁵. The tryptophan residue is essential because chemical modification of it in the protein destroys the activity^{27,28}. Studies of tryptic^{7,16} and peptic peptides^{7,25} suggest that the N-terminal region of this peptide remains intact for the induction of paralysis in guinea-pigs.

Because the sequence Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Arg is effective in inducing an immune response, for EAE to occur this or a closely similar sequence must be exposed in certain areas of the guinea-pig's CNS and it must be accessible to attack by sensitized lymphocytes or by antibody produced locally by them. An ability to induce an autoimmune response is not in itself sufficient to explain the occurrence of paralysis in the injected animal. Two acidic proteins unique to the CNS, the S-100 protein and the 14-3-2 protein, are potent antigens which induce antibodies that can be used to localize the proteins in the glial cells and neurones respectively²⁹. Yet no paralysis is induced in the animals injected with these proteins³⁰. Does the "tryptophan" region of the basic protein have a vital function in certain regions of the CNS? And must the structural integrity of this region be maintained for normal functioning of the CNS?

Neuro-electrical Disturbances in EAE

In rabbits injected with whole brain and adjuvant there was a marked disturbance in the electroencephalogram (EEG) before the onset of clinical systems³¹. It has been shown that γ -globulin from rabbits with EAE but not γ -globulin from normal rabbits, when injected into the ventricle of a recipient rabbit, cause the appearance of high voltage slow waves in its EEG³². Moreover, the basic protein of bovine and human myelin, when injected into the ventricle of a normal rabbit, caused an unexplained electroconvulsive activity³². It has also been shown that serum from animals with EAE and from patients with multiple sclerosis blocked the interneuronal electrical activity of cultures of mammalian brain^{33,34}. Paterson³ has

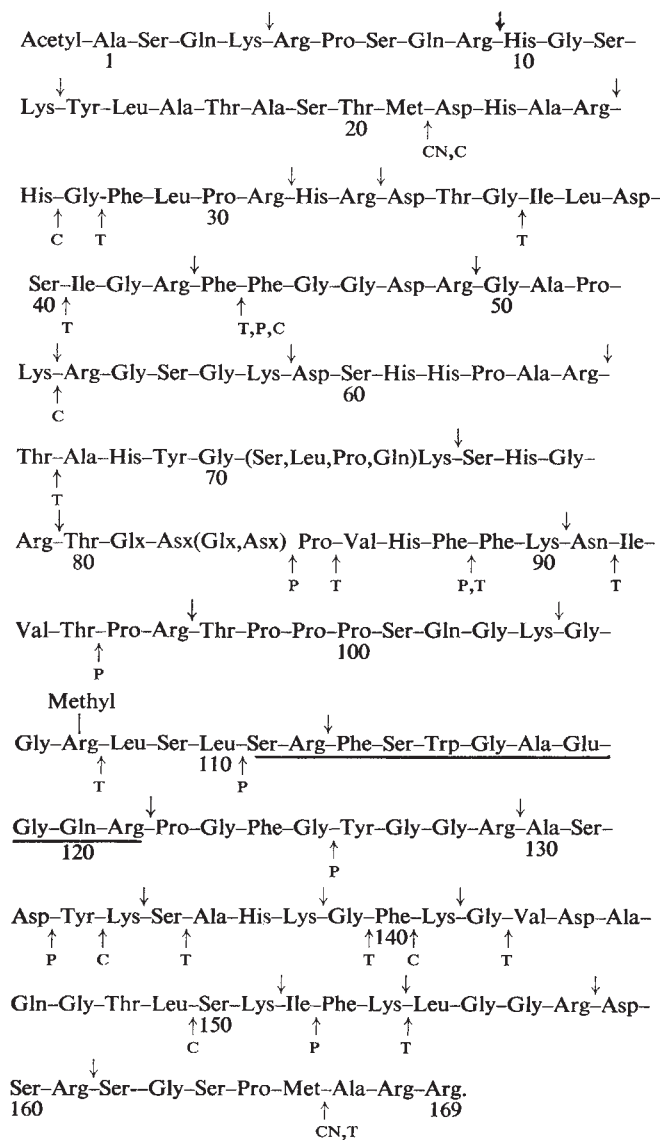


Fig. 1 Amino-acid sequence of the encephalitogenic basic protein from human brain. The encephalitogenic determinant is underlined. Tryptic fission is shown by ↓, points of fission producing overlap peptides obtained from digestion with pepsin, ↑; thermolysin, T; chymotrypsin, C, and cyanogen bromide, CN.

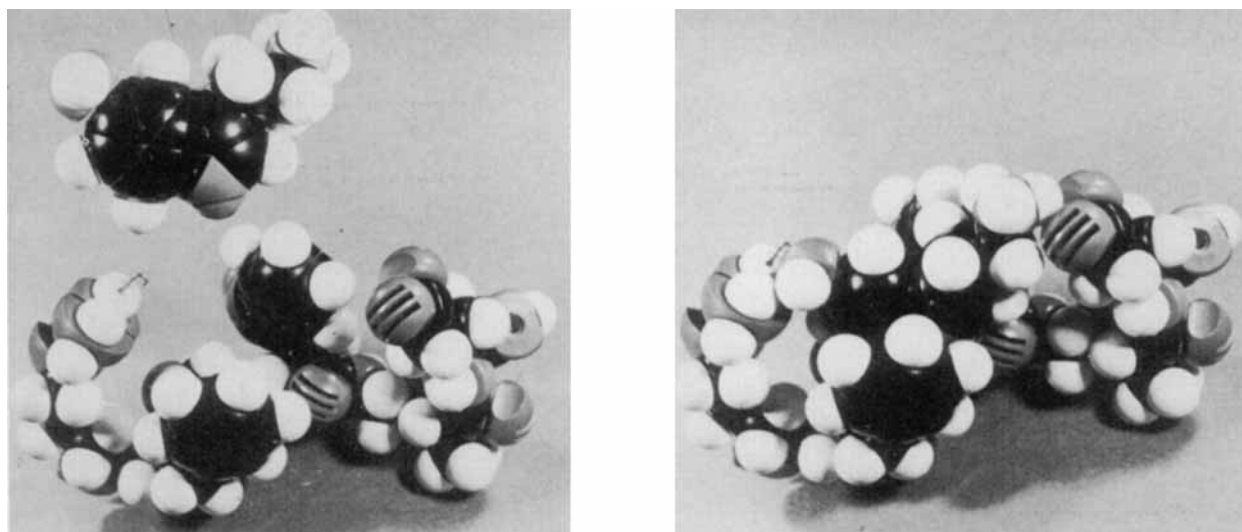


Fig. 2 On the left is a model of part of the principal encephalitogenic determinant Arg-Phe-Ser-Trp-Gly-Ala-Glu from the basic protein of human brain. Above it is 5-HT. On the right 5-HT is shown inserted in this determinant. The determinant is similar in structure to that of a 5-HT receptor site proposed by Smythies, Benington and Morin³⁵.

suggested that the rapidly changing neurological symptoms in EAE and multiple sclerosis "would be easy to understand in terms of altered transmission of electrical impulses across synaptic networks—a physiological dysfunction resulting from antigen-antibody interaction". This raises the question of whether the normal function of the principal encephalitogenic determinant is that of a neuroreceptor molecule in certain regions of the brain.

Receptor Site for 5-Hydroxytryptamine

Smythies, Benington and Morin³⁵ from a detailed consideration of the structure of hallucinogenic antagonists of 5-hydroxytryptamine (5-HT, serotonin) have postulated that a typical binding site for 5-HT in a membrane protein of the CNS would be provided by "two molecules like tryptophan located one above the other so that the 5-HT molecule could intercalate between them. The bonding energy would now be compounded of two π -cloud interactions. . . ." They proposed a triangular shaped binding site where the "ring hydroxyl and indolic N can form hydrogen bonds and the amine group, which will be protonated at a physiological pH, will bond electrostatically. . . ". The first light amino-acids in the encephalitogenic determinant can readily be made to take up such a conformation which can exactly accommodate the 5-HT molecule, with the indole ring sandwiched between the Phe-113 and Trp-115 in the peptide (Fig. 2) and held by a hydrogen bond to the peptide chain and an electrostatic attraction to Glu-118. The OH of 5-HT could form a hydrogen bond with Arg-112, which is between non-polar residues.

Marchbanks³⁶ found a binding site with a high affinity for 5-HT in a synaptosomal preparation. He also showed that there was a binding site of lower affinity associated with monoamine oxidase. Because this high affinity binding site was the only one blocked by D-lysergic acid diethylamide it might be the receptor site for 5-HT in the CNS. Fiszer and De Robertis³⁷ showed that this site was associated with a proteolipid in the membrane fraction of the synaptosomal membranes.

Although the basic protein is a major component of myelin it seems also to be present in synaptosomal membranes. Antiserum from a rabbit immunized with the encephalitogenic basic protein has been shown, by indirect immunofluorescence techniques, to bind to the "1.2 M membrane fraction"³⁷ from human hypothalamus as well as to the myelin fraction (unpublished results of S. Whittingham). In intact myelin of the white matter of the CNS, however, the antigenic determinants

do not seem to be exposed. A basic protein, with the same properties, amino-acid composition and peptide map as the encephalitogenic basic protein³⁸, has been found which does not produce an antiserum which localizes on intact myelin but which does localize on axoplasm of neurones in the spinal cord and on nuclei in other regions of the CNS³⁹. Little basic protein can be extracted from freeze dried myelin unless it is first defatted. Thus it is likely that in the intact myelin *in vivo* the basic protein is buried by lipids.

Myelin could be damaged by immunological attack on the exposed basic protein by lymphocytes (or antibody produced locally by them) in which lytic enzymes are released and cause further exposure of the protein. The functional changes observed in EAE, however, could readily be explained by postulating that the encephalitogenic determinant is a receptor for 5-HT in synaptosomal membranes in certain regions of the CNS.

Possible Block in EAE

I suggest that in guinea-pigs with EAE there is an immune response to a neuroreceptor site for 5-HT which prevents 5-HT functioning as a transmitter substance⁴⁰ in certain regions of the CNS. It is interesting that the 5-HT nerve terminals are in highest concentration in the lumbar and sacral regions of the spinal cord³¹. An immunopharmacological block in this region would account for the localized clinical symptoms such as incontinence and hind quarter paralysis. Moreover, sheep which are poisoned with tryptamine alkaloids from *Phalaris tuberosa* exhibit similar localized clinical symptoms presumably as a result of interaction with the receptor site for 5-HT (ref. 42). In the mid-brain the 5-HT nerve terminals are concentrated immediately under the ependyma in the periventricular grey matter⁴¹ which is a common site for histological lesions in EAE.

A similar immunological block of a 5-HT receptor site in the CNS if it occurred in multiple sclerosis, for which EAE is an animal model³, would explain the temporary functional improvement some patients⁴³⁻⁴⁵ (but not all⁴⁶) experience from treatment with tranlycypromine. This inhibitor of monoamine oxidase would increase the concentration of 5-HT in the CNS and thus make it possible for 5-HT to compete with immunoglobulin for its binding site on the basic protein.

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Plasma Echo and Spin Echo Holophones

by

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A collisionless plasma, or an assembly of nuclear or electron spins in a magnetic field, can function as a holophone which can record a temporal sequence of electrical signals and subsequently play them back when supplied with a cue sequence, consisting of a small part of the recorded sequence.

THE holophone is the temporal analogue of the holograph, in the sense that it stores information, presented to it in the form of a time-sequence, in a temporally non-local manner, just as a holograph stores information presented in the form of a spatial array in a spatially non-local manner. The concept of the holophone was introduced by Longuet-Higgins¹⁻⁴, as a means of understanding certain characteristics of human

memory—particularly our ability to recite a memorized sequence when supplied with a cue consisting of part of that sequence. This concept can be regarded as a development of an earlier suggestion, made by Beurle⁵, van Heerden^{6,7} and Pribram⁸, that the human memory trace should be regarded as in some sense analogous to a three dimensional hologram.

In addition to its importance in the context of the theory of the brain, the holophone is also of practical interest. Two applications immediately suggest themselves: a holophone could act as a temporal pattern recognition device (for example, to recognize human speech or to search for the presence or absence of a particular cue sequence in a previously stored sequence of much greater length) or as a content addressable computer memory, capable of reciting the information associated with—for example, following—a given information sequence. Exactly analogous roles have been proposed for holographs, and in both cases the same difficulties arise. When used for pattern recognition, the device is (or can be made^{2,6}) reliable but is slow; used for information recitation, the device is fast but unacceptably noisy. In particular, pattern recognition by a holophone using the "spike" method² is no faster

than a sequential search of the same information by a conventional computer. The recitation approach is potentially much faster, because there is no delay between the end of the cue and the start of the recitation, but according to present theory the recited sequence would be unrecognizable because the signal to noise ratio is^{2,6} of the order of C/R , where C and R are the amounts of information in the cue and in the memory record respectively.

Previous work has not made clear how far these are difficulties of principle and how far they are merely features of the particular implementations of the holograph and holophone concepts which have been considered. The only implementations of the holophone which have been described in the literature—those of Longuet-Higgins² and Gabor⁹—are not plausible practical devices for recording information sequences of interesting length at reasonable speed and (so far as I know) have never been constructed. Their behaviour has been simulated on a conventional computer² by Willshaw and Longuet-Higgins; as they point out, however, the simulation is necessarily so voracious of computer time that this cannot be regarded as an acceptable alternative implementation. Thus alternative (and, it is to be hoped, more practical) implementations of the holophone concept may be of interest.

This article proposes two new implementations. I shall first show that a collisionless plasma, excited by a sequence of spatially periodic electrostatic pulses, behaves as a holophone in the above sense. It is well known that a plasma responds to a sequence of two such pulses by producing a sequence of "echo" pulses at certain later times. I shall now consider the consequences of applying a sequence of N pulses of varying amplitude, at times $\tau_1, \tau_2, \dots, \tau_N$, which for simplicity I shall suppose to be equally spaced. The treatment follows that of O'Neil and Gould¹⁰, section IV. The motion of a single particle in this electric field is determined by

$$\ddot{x} = \sum_{i=1}^N E_i \delta(t - \tau_i) \sin kx \quad (1)$$

where $E_i \equiv eE_i/m$ is the amplitude of the i th pulse and k is the wave number of the spatial periodicity of the pulse, which for simplicity I assume to be large compared with k_D , the reciprocal Debye length, so that coherent plasma effects can be ignored. The solution of (1) is

$$x(t) = x_0 + v_0 t + \sum_i E_i (t - \tau_i) \Theta(t - \tau_i) \sin kx_i \quad (2)$$

where

$$x_i \equiv x(\tau_i) = x_0 + v_0 \tau_i + \sum_j E_j (\tau_i - \tau_j) \Theta(\tau_i - \tau_j) \sin kx_j$$

and $\Theta(t)$ is the Heaviside step-function.

It can be seen from equation (2) that $x(t)$ is a strongly non-linear function of the amplitudes E_i . In what follows I assume that these amplitudes are small, in the sense that the change in particle velocity after N pulses is small compared with the plasma thermal velocity, and consider expansions of $x(t)$ in increasing powers of the amplitudes E_i . It is convenient to define

$$\varphi \equiv kx_0 \quad \alpha \equiv kv_0 t \quad \alpha_i \equiv kv_0 \tau_i \quad \varepsilon_i \equiv kE_i (t - \tau_i) \Theta(t - \tau_i) \\ \varepsilon_{ij} \equiv kE_j (\tau_i - \tau_j) \Theta(\tau_i - \tau_j) \quad (3)$$

Then equation (2) can be written in the iterated form

$$kx(t) = \varphi + \alpha + \sum_i \varepsilon_i \sin(\varphi + \alpha_i + \sum_j \varepsilon_{ij} \sin(\varphi + \alpha_j + \sum_k \varepsilon_{jk} \sin(\varphi + \dots))) \quad (4)$$

To determine the response of an initially Maxwellian plasma to this pulse sequence the perturbed charge density $\rho(x, t)$ is calculated using Liouville's theorem as in ref. 10. It is clear from parity and symmetry considerations that $\rho(x, t)$ is an even periodic function of x : thus

$$\rho(x, t) = 2e \sum_{n=1}^{\infty} \cos(nkx) P_n(t) \quad (5)$$

and

$$P_n(t) = P_{-n}(t) = \int_{-\infty}^{\infty} f_0(v_0) \frac{1}{2\pi} \int_0^{2\pi} e^{inkx(\varphi, v_0, t)} d\varphi dv_0 \\ \equiv \int_{-\infty}^{\infty} f_0 e^{in\alpha} F_n dv_0 \quad (6)$$

where

$$f_0(v_0) = \frac{1}{(\pi v_T)^{1/2}} e^{-(v_0/v_T)^2} \quad (7)$$

To evaluate F_n , the exponential e^{inkx} is expanded making repeated use of the identity

$$e^{i\varepsilon \sin \varphi} = \sum_{m=-\infty}^{\infty} J_m(\varepsilon) e^{im\varphi} \quad (8)$$

This leads to a sequence of products of sums over Bessel functions which can be written in the form:

$$F_n = \frac{1}{2\pi} \int_0^{2\pi} e^{in\varphi} \prod_n \prod_i^{N-1} (1 + S_i \prod_j^{i-1} (1 + S_{ij} \prod_k^{j-1} (1 + S_{ijk} \dots))) d\varphi \\ = \frac{1}{2\pi} \int_0^{2\pi} e^{in\varphi} (1 + \sum_i S_i + \sum_i \sum_j S_i S_j + \sum_i \sum_j \sum_k S_i S_j S_k + \dots) d\varphi \\ + \sum_i \sum_j [\sum_k S_i (S_j S_k + S_j S_{jk} + S_{ij} S_{ijk}) + \sum_k S_i S_j S_{ik}] + O(S^4) \dots d\varphi \quad (9)$$

where

$$\prod_n = \prod_i^N J_0(n\varepsilon_i)$$

$$S_i \equiv \sum_{m_i \neq 0} \frac{J_{m_i}(n\varepsilon_i)}{J_0(n\varepsilon_i)} \left[\prod_j^{i-1} J_0(m_i \varepsilon_{ij}) \right] e^{im_i(\varphi + \alpha_i)} \text{ etc.}$$

and hence, performing the φ and v_0 integrations, an expression is obtained of the form

$$P_n(t) = \prod_n [\delta_{n,0} + \sum_i \sum_{m_i \neq 0} A_{m_i} g(nt + m_i \tau_i) \delta_{n+m_i,0} \\ + \sum_i \sum_j \sum_{m_i, m_j \neq 0} B_{m_i m_j} g(nt + m_i \tau_i + m_j \tau_j) \delta_{n+m_i+m_j,0} \\ + \sum_i \sum_j \sum_k \sum_{m_i, m_j, m_k \neq 0} C_{m_i m_j m_k} g(nt + m_i \tau_i + m_j \tau_j + m_k \tau_k) \delta_{n+m_i+m_j+m_k,0} \dots] \quad (10)$$

where $g(t) = \int_{-\infty}^{\infty} f_0(v_0) e^{ikv_0 t} dv_0$ and the coefficients A , B and C

are combinations of Bessel functions. The function $g(t)$ is an echo pulse shape factor which for $k v_T \Delta t \gg 1$ (where $\Delta t = \tau_{i+1} - \tau_i$) is sharply peaked about $t=0$ and is negligible for $t \gtrsim \Delta t$. Thus the g factors and the Kronecker δ -functions ensure that only a selection of the terms in equation (10) contribute to $\rho(x, t)$. In particular for $t > \tau_N$, the first two terms contribute nothing; in the third term, only those elements with $|m_i| + |m_j| > |n| + 1 \geq 2$ can contribute; in the fourth term those with $|m_i| + |m_j| + |m_k| > |n| + 1$. Because for small ε

$$J_m(\varepsilon) = \frac{(-1)^{|m|}}{|m|!} \left(\frac{\varepsilon}{2} \right)^{|m|} (1 + O(\varepsilon^2))$$

there are no terms of order less than ε^3 , and in this order

$$P_n = P_1 = \frac{k^3}{8} \sum_i \sum_j \sum_k^{i-1} \frac{E_i E_j E_k}{1 + \delta_{ij}} (t - \tau_i)^2 g'(t - \tau_i - \tau_j + \tau_k) \quad (11)$$

where $g'(t) = tg(t)$, and is a pulse shape factor similar to the first derivative of the Dirac δ -function. Because this is negligible unless t is close to some integral multiple of $\Delta\tau$, say τ_i , equation (11) can be written as

$$P_1 = \frac{k^3}{8} g'(t - \tau_i) \sum_{i=1}^N \sum_{j=1}^{I-1} \sum_{k=1}^{J-1} \frac{E_i E_j E_k}{1 + \delta_{ij}} (\tau_i - \tau_i)^2 \delta_{i+k-i-j} \quad (12)$$

I shall now suppose that the sequence E_i is made up of two parts—a “record” sequence R for $i=1, 2 \dots I$, followed by a “cue” sequence C for $i=I+1, I+2 \dots I+J=N$, after which the applied signal is zero; then a “playback” sequence is obtained, of which $P_1(\tau_i)$ is a typical element. If C is uncorrelated with R the playback has a purely noise character: if the cue coincides with some part of the record sequence, however (that is, if for some C , $E_{I+i} = E_{C+i}$, $i=1, 2 \dots J$), there is constructive interference between the echoes, and the playback consists of a rather noisy recitation of the remainder of the recorded sequence. For example, if the amplitudes $E_i = \pm 1$ and the signal is of zero mean, then for $2I - C > I > N$

$$P_1(t) = \frac{k^3}{8} g'(t - \tau_i) [E_{I-I+C} \sum_{k=1}^J (\tau_{I-I+C} - \tau_k)^2 + \text{Noise}] \quad (13)$$

If the amplitudes E_i are normally distributed, the signal to noise ratio is readily estimated as $J \tau_{I-I}^2 / N \tau_N^2$, in qualitative agreement with ref. 2 (and in disagreement with ref. 9).

I shall now consider a second implementation, based on the phenomena of “spin echoes”, first demonstrated by Hahn¹¹ in nuclear spin resonance experiments, and subsequently shown¹²⁻¹⁴ to occur in electron spin resonance experiments in various solid-state systems. In these experiments, a sequence of two or three radio frequency pulses is applied at a frequency ω close to the nuclear or electron spin resonance frequency Ω in an external magnetic field. Because of slight non-uniformities in the magnetic field, there is a spread in the values of $\Delta\omega \equiv \omega - \Omega$ and the spin population is distributed over these values with a probability

$$P(\Delta\omega) = \frac{T_2^*}{(2\pi)^{1/2}} e^{-\frac{1}{2}(\Delta\omega T_2^*)^2}$$

The theory of the response of such a system to a sequence of three equal radio frequency pulses was given by Hahn¹¹; his results are readily generalized to three unequal pulses, with $\gamma \tilde{H} t_{\omega} = \xi_1, \xi_2, \xi_3$ at times τ_1, τ_2, τ_3 (where $\tilde{H}$ is the amplitude of the radio frequency magnetic field, t_{ω} is the time for which it is applied, assumed to be much less than the interval between pulses, and γ is the appropriate gyromagnetic ratio). For times which are short compared with the spin-spin and spin lattice relaxation times, the response is

$$\begin{aligned} V(t) = & \frac{1}{4} [\sin \xi_1 (1 - \cos \xi_2)(1 + \cos \xi_3) h(t - 2\tau_2 + \tau_1) \\ & + \sin \xi_1 (1 + \cos \xi_2)(1 - \cos \xi_3) h(t - 2\tau_3 + \tau_1) \\ & + 2 \cos \xi_1 \sin \xi_2 (1 - \cos \xi_3) h(t - 2\tau_3 + \tau_2) \\ & + 2 \sin \xi_1 \sin \xi_2 \sin \xi_3 h(t - \tau_3 - \tau_2 + \tau_1) \\ & - \sin \xi_1 (1 - \cos \xi_2)(1 - \cos \xi_3) h(t - 2\tau_3 + 2\tau_2 - \tau_1)] \end{aligned} \quad (14)$$

where $h(t) \equiv \int_{-\infty}^{\infty} \cos(\Delta\omega) P(\Delta\omega) d\Delta\omega$. The first three terms in

this expression represent the sum over the two-pulse echoes evoked by all the pairs of pulses in the sequence; the remaining terms are those which depend on the sequential occurrence of three pulses. The generalization to a sequence of N pulses will clearly involve N similar sums: if the ξ are small, however, and terms of order ξ^3 only are retained, the result becomes

$$V(t) = \frac{1}{2} \sum_{i=1}^N \sum_{j=1}^{i-1} \sum_{k=1}^{j-1} \frac{\xi_i \xi_j \xi_k}{1 + \delta_{ij}} h(t - \tau_i - \tau_j + \tau_k) \quad (15)$$

This expression is very similar to equation (11), and an analogous argument shows that if the signals ξ_i are information-bearing, the response is again holophonic, with a signal to noise ratio of order J/N .

It seems a reasonable speculation that there will be other implementations, based on other echo phenomena in which the echo amplitude depends on the signal amplitude in a sufficiently non-linear manner. As Gabor has pointed out, the essential requirement is that the system shall be capable of simulating the operations of shift, multiplication and summation.

The rather unfavourable signal to noise ratio found for both the implementations of the holophone discussed here (as well as that of Longuet-Higgins) should not be used to dismiss them as impracticable devices or as irrelevant as models of the human brain. The playback is highly susceptible to “cleaning up” procedures, because, as Gabor⁹ observed, the probability of a playback sequence with the amplitudes consistently given by the first term in equation (13) resulting from chance is very small. In computer simulations based on equation (15) (chosen in preference to equation (12) because the modulation factor $(\tau_i - \tau_i)^2$ is absent) I have found that a great improvement can be obtained simply by attaching significance to the sign of $V(t)$ and not to its magnitude. For $J=10$, $N=100$ this leads to a 70% agreement between the recorded and playback sequences. More sophisticated “clean up” procedures could clearly improve the playback clarity further; the effectiveness of this simple non-linear element in the output circuit, however, suggests that non-linear processes of higher order than the cube of the signal strength within the holophone itself might play a similar part.

A second feature which might be held to be a disadvantage of all except Gabor's implementation is the existence of an upper limit to the amount of stored information. In Longuet-Higgins's device this is set by the band width of the filters; in the two implementations considered here the limit is set by the relaxation time, determined by collisions in the plasma and self-diffusion and/or interactions of the spins respectively, in relation to the shortest feasible pulse length. The practical limit is probably in the region of 10^3 – 10^5 pulses^{12,15}. It should be remembered, however, that there is no simple mechanism for stopping the playback of these holophones once initiated, so in any practical application it is likely that they would be operated in conjunction with a backing store, used to reprime the holophone memory, which would periodically be allowed to lapse. Thus short relaxation times are not necessarily disadvantageous. Clearly a desirable feature, which none of the implementations which I have mentioned possess, would be for the back-up storage system to be within the holophone mechanism, and capable of repriming the holophone memory instantly. It is tempting to speculate whether neural networks could implement such a concept.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Energy Spectrum of a Nova-like X-ray Source

A NEW X-ray source was born near or in the Centaurus constellation during July 6-9, 1969 (see refs. 1 and 2). In a few days the source reached a maximum intensity that was approximately twice that of Sco X-1. The intensity remained nearly constant for about two months after which it decreased to approximately a half of its maximum. The source became dim gradually from early September and unobservable at the end of September. The hardness of the spectrum represented by the ratio of counts over 6-12 keV to counts over 3-6 keV had kept constant after the source passed the first phase of its development. We have reported³ the measurement of the source in the energy range 2-20 keV performed with well calibrated proportional counters on board a sounding rocket, Kappa-9M, launched on August 7, 1969, from KSC (Kagoshima Space Center, 131° 04' 45" E, 31° 13' 00" N).

We now describe the results of further analysis of the data obtained in the same flight and the succeeding flight by the rocket, S-210, launched on the next day from the same place. We took special care in the fabrication and calibration of the X-ray detectors, and in the aspect analysis of the rocket, in order to minimize systematic errors in derived energy spectra. The spectrum obtained suggests a thermal origin for the source with a strong gas absorption of the flux in the energy range <3 keV indicating the existence of a dense circumstellar gas.

Each of the rockets carried a pair of identical proportional counters with electronic circuits (counters A and B). The

counters, filled with 300 torr Xe plus 10% methane gas, had thin beryllium windows each of 38 cm² effective area and were equipped with slat collimators which limit the fields of view of the detectors. The field of view was 7° FWHM in azimuth and extended from -13° to +55° in elevation of the rocket frame of reference. The long direction of the field of view of counter A was parallel to the rocket spin axis and that of counter B was tilted by 25°.

The set of two counters was surrounded by a plastic veto counter, except on the window side, to reduce spurious counts due to the corpuscular radiation. The detectors scanned the sky by means of the rocket spin. The time and the amplitude of each pulse produced in the counters were telemetered to the ground.

The counters were equipped with beryllium windows of approximate thickness 108 μ m. The thickness was made uniform for total area of the window and equal for all counters with regard to the absorption of X-rays to the precision of ± 4 μ m equivalent of beryllium by means of compensation with thin Mylar films. The calculated efficiency of the counters was confirmed by K X-rays of Al, S, Cu and Ag. The ⁵⁵Fe X-ray source produced a pulse height distribution with a resolution of 20% FWHM. The ⁵⁵Fe source introduced in the field of view of the counter once every 36 s confirmed the stability of the system throughout the flight.

The rocket, Kappa-9M, was launched on August 7, 1969, at 1215 UT from KSC. It was spin stabilized at approximately 2 revolutions s⁻¹ and the apogee was at an altitude of 330 km. The X-ray observation was performed for approximately 500 s. The second observation was performed with the rocket S-210 launched on August 8, 1969, at 1200 UT. Its spin rate was approximately 2 revolutions s⁻¹ and the apogee was 101 km.

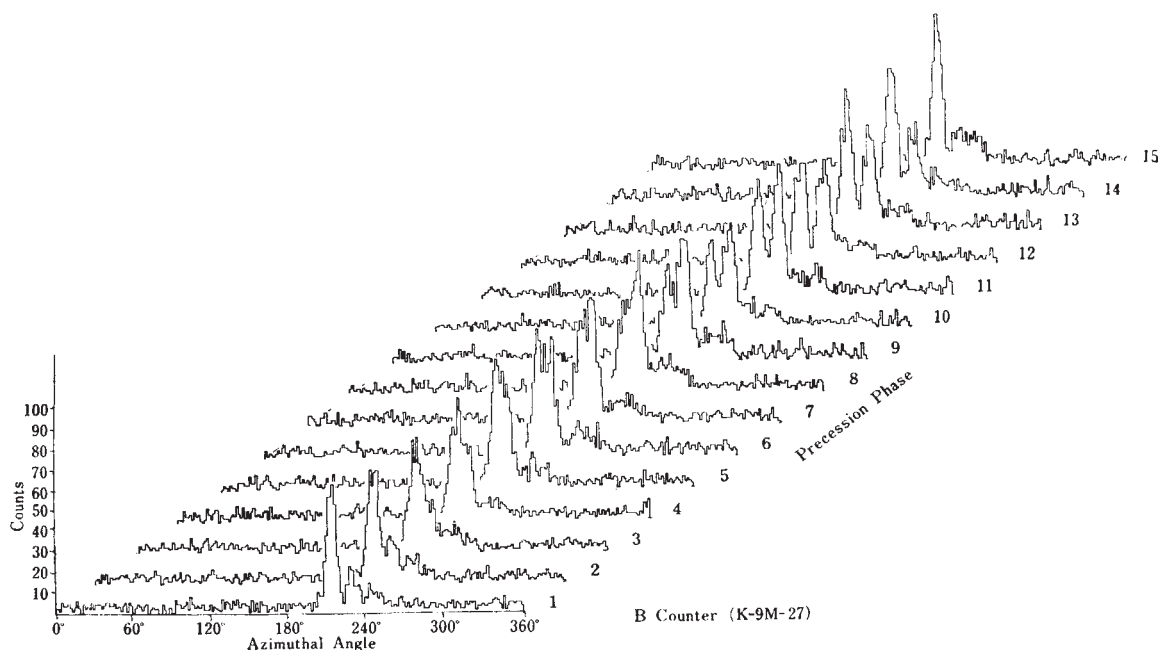


Fig. 1 Count rates of counter B on board Kappa-9M-27 against relative rocket azimuth grouped for 15 intervals of the precession phase.

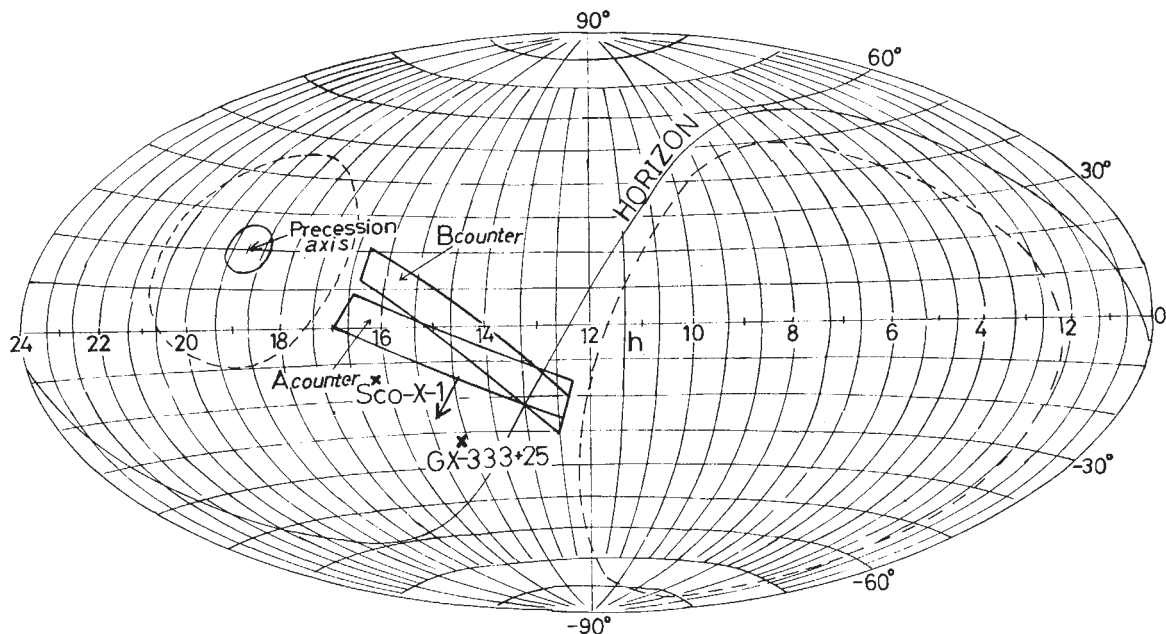


Fig. 2 The boundary of the scanned region in the sky is indicated by broken lines on the celestial sphere (right ascension and declination). The precession cone is shown by the small circle. Full fields of view of counters A and B are shown by squares.

The spin period and the precession period of the freely spinning rocket were determined by means of the geomagnetic aspectometer and counts of X-rays were accumulated against the relative rocket azimuth. Fig. 1 shows an example of a diagram representing count rates against relative azimuths grouped for 15 intervals of the precession phase. Two distinct peaks on the histogram for a certain range of the precession phase are due to Sco X-1 and the new source.

By analysis of the output of the geomagnetic aspectometer, the angle between the geomagnetic lines of force and the precession axis, and the coning angle of the precession, were obtained. Also the relative azimuthal phase of Sco X-1 acquisition by counters A and B was analysed, and the angle between the direction of Sco X-1 and the precession axis, and the coning angle of the precession, were derived. The precession axis was determined as one of two intersections of the two small circles on the celestial sphere centred at the direction of geomagnetic line of force and of Sco X-1. One of the two intersections was chosen from knowledge of the rocket trajectory. The aspect of the rocket was thus obtained with an accuracy of approximately 1° throughout the flights above 90 km.

The scanned region of the sky in celestial coordinates is shown in Fig. 2 for Kappa-9M-27, and the location of the new source corresponding to the second peak in Fig. 1 is plotted on the celestial sphere.

Pulse height spectra for each of the two sources were produced from the observed data with the contribution of the background component subtracted. The energy spectrum analysis was performed by comparing integral transforms of an assumed energy spectrum expressed by a few variable parameters with the obtained pulse height spectrum. Various factors such as the detector efficiency, the pulse height resolution and background subtraction are included in the integral transforms.

The location of the source was determined as follows

$$\begin{aligned}\alpha &: 14^{\text{h}} 46 \pm 8^{\text{m}} \\ \delta &: -30^\circ 20' \pm 1^\circ 30'\end{aligned}$$

The error box of this location includes the borderline between the Centaurus and Hydra constellations. The source was, therefore, GX 333+25 after its approximate galactic coordinates³.

Energy spectra of the source and Sco X-1 obtained during

the two rocket observations are summarized in Fig. 3. The model spectra of thermal radiation emitted by a thin hot plasma with an appropriate temperature are indicated by the solid lines. It is seen that the spectrum is well represented by a thermal spectrum in the energy range 4–20 keV. Below 4 keV the observed spectrum deviates from the thermal spectrum. The deviation is considered to be real, because it is not apparent in the observed spectrum of Sco X-1 simultaneously obtained. The broken lines indicate the effect of absorption in cool material calculated by means of absorption cross sections derived by Bell and Kingston⁴. From a comparison of the data with the calculation, a columnar density of the cool material of $3 \times 10^{22} \text{ cm}^{-2}$ is derived.

By analogy with Sco X-1 the appearance of a 12–13 magnitude optical object or even a nova outburst could be expected to accompany the sudden appearance of such a hot plasma, but no definite optical identification of the source has been made (private communication from W. A. Hiltner).

The energy spectrum suggests the thermal origin of the source with a considerable absorption. The strong absorption

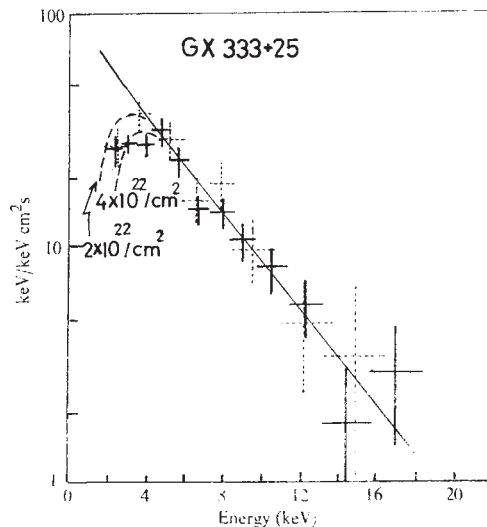


Fig. 3 Energy spectra of the source obtained during the rocket observations. In view of relatively low apogee of S-210-2 (August 8, 1969) the atmospheric attenuation is corrected. —, August 7, 1969; ---, August 8, 1969.

reported for a few sources at low galactic latitudes has so far been explained in terms of interstellar gas. Because of the high galactic latitude of the present source, however, the estimated columnar density of cool material, $3 \times 10^{22} \text{ cm}^{-2}$, is anomalously high. According to the compiled results of 21 cm observations the columnar density of atomic hydrogen along the line of sight towards the general direction of the source is approximately 10^{21} cm^{-2} (E. Daltabuit, private communication). This is the upper limit of the number of hydrogen atoms in interstellar space. The absorption is, therefore, concluded to be caused by a circumstellar gas unless it is explained in terms of an unexpected amount of hidden gas like hydrogen molecules. Whether the existence of the dense circumstellar cloud has any genetic relation to the sudden appearance of this source is open to question. If this cloud also contains dust, the fact that the source had not been optically observed may be reasonably understood. It has been noted² that the time profile of the variation of this source is similar to that of Cen X-2. The latter, however, did not exhibit anomalous absorption⁵.

The sudden appearance of two sources in the past few years makes it reasonable to expect the birth of more sources, and there is no doubt that the investigation of the newborn sources will present an important clue to deeper understanding of the X-ray source. The value of continuous survey of the X-ray sky is obvious.

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Identity of a Common Contaminant of Apollo 11 Lunar Fines and Apollo 12 York Meshes

THE likelihood of organic contamination of the lunar samples obtained in the Apollo missions has been pointed out repeatedly¹⁻⁴. Rocket exhaust, terrestrial atmosphere and those manufactured materials which might have come in contact with the lunar samples at one time or the other during collection, transport, preliminary examination, and distribution are the generally accepted sources for contamination introduced previous to analysis. One of the materials being used in the programme is the York mesh^{3,4}, used as a packaging aid in the Apollo lunar sample boxes.

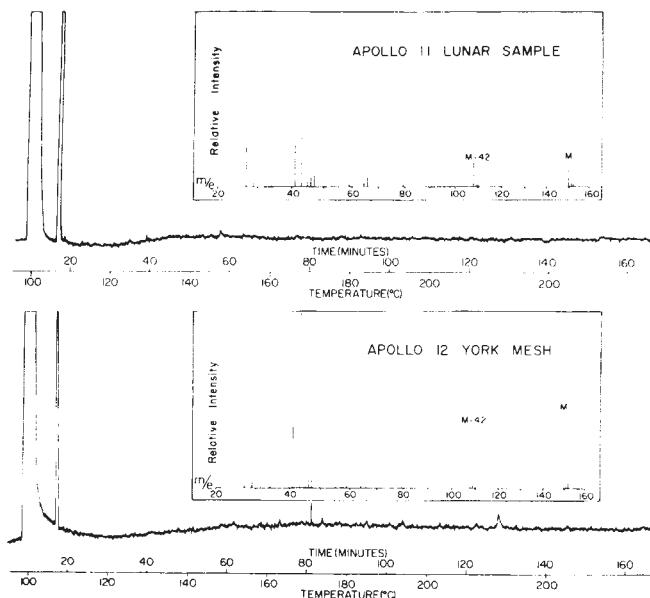


Fig. 1 Gas chromatograms and mass spectra of diisopropyl disulphide extracted in both lunar samples from Apollo 11 and York mesh from Apollo 12. The gas chromatograms were obtained in an F and M 880 gas chromatograph on 120 m $\times$ 0.76 mm i.d. capillary column coated with 'Polysev' and operated at $1.2 \mu\text{g}/\text{cm}^2$ of helium, programmed from 100°C to 200°C at a rate of $2^\circ \text{C}/\text{min}$. The mass spectra were recorded at 70 eV as the effluent from the same capillary column was introduced to an LKB 9000 gas chromatograph-mass spectrometer under the same chromatographic conditions.

In our analysis of the lunar samples from Apollo 11, we reported on the extraction of a single compound, presumed to be a contaminant^{5,6}. When the same analytical techniques used for the lunar fines were applied to the analysis of Apollo 12 York mesh samples, this same single organic compound was detected. Fig. 1 shows the gas chromatograms and mass spectra for these analyses. As illustrated, the compound in question is diisopropyl disulphide. Briefly, the analytical procedure included solvent extraction [benzene/methanol (3:1)] in an all glass 'Soxhlet' apparatus with an interchangeable sintered glass cup used as a sample holder, followed by gas chromatographic and mass spectrometric analyses. We used high resolution open tubular capillary columns coated with suitable phases and an LKB 9000 gas chromatograph-mass spectrometer. Details of the analysis have been given elsewhere^{5,6}. In this case duplicate extractions were carried out by two independent analysts on portions of the same sample of lunar material, and five different pieces of York mesh. Any contamination introduced during analysis would be easily identified as a result of the duplicate extractions. Furthermore, this compound was not observed in any of the blanks and control runs, solvents and the sample container including the 'Teflon' liner.

Two sets of extractions were performed on the Apollo 11 lunar samples. Amounts of 0.123 g and 0.148 g of lunar fines were analysed in the first set, while in the second set we extracted 1.105 g and 1.492 g. Diisopropyl disulphide was detected only in the first set of extractions which were aliquots removed from the top of the original sample container (closed with an aluminium screw cap lined with a 'Teflon' sheet) filled with 104.7 g of lunar fines No. 10086,16. We did not find this compound in the second set of extractions which were removed from the bulk of the sample near the bottom of the container. The amounts of contamination calculated for this single contaminant are $13.70 \mu\text{g}/\text{g}$ for the 0.123 g extraction and $9.65 \mu\text{g}/\text{g}$ for the 0.148 g aliquot. An apparent average contamination value of $1.07 \mu\text{g}/\text{g}$ (or 1 p.p.m.), which we reported previously^{5,6}, is obtained if the total amounts of contamination detected are averaged over the total of 2.868 g

of lunar sample extracted. These results indicate the highly heterogeneous distribution of this contaminant. Diisopropyl disulphide has been detected during analysis of Apollo 11 samples by at least one other group of investigators⁷ in extractions carried out by a method similar to ours. But the compound was not detected by this same group in volatilization experiments performed in the direct probe of a high resolution mass spectrometer. These investigators⁷ pointed out the possibility that the diisopropyl disulphide could have originated by solvent impurities like isopropyl alcohol reacting with metal sulphides present in the sample. Even though this reaction is a possibility which cannot be entirely ruled out, the fact that this compound was not found in all four extracts of the lunar fines indicates that it may come from other sources. The heterogeneous distribution of the compound in the lunar sample and its presence in the York mesh indicate that this packaging material, used in the boxes of both the Apollo 11 and 12 missions, may be the source of contamination. The Apollo 12 York mesh was prepared for use in the mission by the same procedures used for the Apollo 11 York mesh. Other workers⁸ have also identified this compound in Apollo 12 meshes by application of high resolution mass spectrometry to similar extracts.

It is not yet possible to say at what point this contaminant is introduced to the sample. The compound may have remained on the York mesh after cleaning and have been transferred to the lunar sample, or a third source such as a processing cabinet may have contaminated both the lunar sample and the York mesh. Subsequent monitoring samples to be provided by NASA may answer these questions.

The presence of such a contaminant in the lunar fines may jeopardize certain measurements but the majority are essentially unaffected. Specific measurements which this contaminant may have influenced are the determinations for total carbon and sulphur, as well as the isotopic ratios of both elements. Because of its heterogeneous distribution the quantitative effect of this contaminant on the carbon and sulphur values obtained by other investigators⁸⁻¹⁰ is difficult to estimate. The carbon content and stable isotopic values obtained in our measurements^{5,6}, however, would not be significantly affected, for we used samples of 5 g–10 g. Such large samples would approximate the bulk average contamination value of 1 p.p.m. which is less than 1% of the average total carbon content of our samples (≈ 125 p.p.m.). This small contribution is not sufficient to explain the differences in the $\delta^{13}\text{C}$ values reported⁹⁻¹¹.

Other investigators^{7,12} have also reported various degrees of contamination in their Apollo 11 samples. Overall values were estimated to be about 10 p.p.m.¹³ which is in line with the results given above considering they refer to the single compound extracted from the top layer of our sample.

We thank Dr R. C. Murphy for Apollo 12 York mesh coupons, D. W. Nooner for performing duplicate extractions and gas chromatographic analyses, and H. Liebig for helpful comments. This work was supported by the US National Aeronautics and Space Administration.

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New Class of Gravitational Wave Detectors

In a series of classic articles, Weber¹⁻³ derived the equations of motion and the response of mass quadrupole detectors to tensor gravitational waves. He has also constructed a detector which is a cylindrical rod tuned to its fundamental longitudinal acoustic resonance. These devices (class 1 detectors) have a definite relationship between the resonant frequency and the dimensions, in consequence of which they become very large (and expensive) at low frequencies. Using a somewhat arbitrary criterion (discussed later), one may state that class 1 detectors are difficult to build at frequencies below 1,000 Hz. There are other mechanically resonant devices (for example, tuning forks, rings and hollow "squares"), which we call class 2 detectors, which have a different relationship between the resonant frequency and their dimensions. Application of the same arbitrary criterion to class 2 detectors establishes that their useful frequency range can be extended to ~ 30 Hz and that their sensitivity at the lower frequencies is comparable with class 1 detectors. Thus class 2 detectors are useful in a frequency range not easily accessible to class 1 detectors.

The equation of motion of both class 1 and class 2 detectors is easily shown to be (under suitable orientation)

$$\ddot{\xi} + \frac{\omega_0}{Q} \dot{\xi} + \omega_0^2 \xi = -c^2 B l \epsilon^{tot} \quad (1)$$

where B is the amplitude of the Riemann curvature tensor describing the (sinusoidal) wave, l is the "size" of the resonator, ξ is the displacement from equilibrium, ω_0 is the fundamental resonant (angular) frequency and Q is the usual quality factor describing the damping. The relationship between the total Q , the internal Q_{in} (internal damping) and the external Q_{ex} (loading) is $Q^{-1} = Q_{in}^{-1} + Q_{ex}^{-1}$.

The steady state solution to equation (1) for the amplitude of ξ for waves with $\omega \approx \omega_0$ is

$$|\xi| = Q \frac{c^2 B l}{\omega_0^2} \quad (2)$$

In order to detect the strains or displacements, the power received must be delivered to an external recording instrument. The power P available to this instrument is given by¹⁻³

$$P = (k M \omega_0^2 \xi^2) / 2 Q_{ex} \quad (3)$$

where M is the mass and k is a constant of the order of unity which depends on the shape of the detector. The maximum power P_{max} is delivered to the recording instrument when $Q_{ex} = Q_{in}$

$$P_{max} = \frac{k}{8} \frac{c^4 B^2 M l^2}{\omega_0} Q_{in} \quad (4)$$

where the value of $|\xi|$ from equation (2) has been used.

At this point it is convenient to use a relationship given by Weber¹⁻³ between the energy flux S and the amplitude B of the gravitational wave (under the condition of negligible relative velocity of the source and antenna)

$$B^2 = \frac{4\pi G \omega_0^2}{c^7} S \quad (5)$$

Equation (4) can now be written

$$P_{\max} = k \frac{\pi}{2} \frac{G}{c^3} Q_{\text{in}} A S \quad (6)$$

where we have collected the quantities ω_0 , M , l into the factor

$$A = \omega_0 M l^2 \quad (7)$$

which is closely related to the cross section for absorption of energy from the gravitational wave. It is seen that, in the design of a detector, A is the only parameter that can be varied substantially and it is the parameter that distinguishes one gravitational detector from another. (It is assumed that the Q , which depends only on internal hysteresis of the material and similar factors, is already at its practical limit.) The quantity A cannot be made arbitrarily large for a particular choice of ω_0 because there is a functional relationship between ω_0 and the dimensions of the detector. It is at this point that we define the classes of detectors.

$$\text{Class 1 : } \omega_0 \propto l^{-1}$$

$$\text{Class 2 : } \omega_0 \propto l^{-2}$$

For class 1 detectors the relationship between ω_0 and the length l_1 is (we will use as an example a bar of length l_1 and square cross section $d \times d$)

$$\omega_0 = \frac{\pi v_s}{l_1} \quad (8)$$

where v_s is the velocity of sound. The mass is given by

$$M = \rho d^2 l_1 = \rho \eta_1^2 l_1^3 \quad (9)$$

where ρ is the density and η_1 is the ratio of d to l_1 . The quantity A_1 is thus found to be

$$A_1 = \frac{\pi}{16} v_s^5 \rho \eta_1^2 \frac{1}{f_0^4} \quad (10)$$

where in equation (10) we have used the relation $\omega_0 = 2\pi f_0$. At fixed frequency, A_1 can be increased only by increasing η_1 (v_s and ρ are fixed because the material is chosen to maximize Q_{in}). The practical limit is $\eta_1 \sim 0.5$. The figure shows $A_{1,\max}$ for aluminium (all the calculations here are based on aluminium) ($v_s = 6.4 \times 10^5 \text{ cm s}^{-1}$, $\rho = 2.7 \text{ g cm}^{-3}$) as a function of f_0 and as a function of l_1 . A becomes very large at low frequencies. At low frequencies, however, these detectors also are very large. If the arbitrary condition is adopted that detectors larger than 300 cm are difficult to make, then this would restrict class 1 detectors to frequencies above 1,000 Hz and to values of A less than 10^{16} . It is interesting that Weber's (class 1) detector is nearly at these limits; $f_0 = 1,660 \text{ Hz}$, $A_1 = 3.4 \times 10^{14}$.

Because all the class 2 detectors behave in the same way, to be specific we consider the vibration of the "hollow" square. The vibrational mode that concerns us is the quadrupole mode where two opposite sides move in and out in phase with each other but opposite in phase to the other two sides. (This is the lowest mode. The next highest mode is the "scalar" mode where all 4 sides go in and out together in phase. Thus class 2 detectors can also detect scalar gravitational radiation if it exists.) The vibrational frequency in the quadrupole mode is approximately that of bending of a beam with pinned (pivoting)

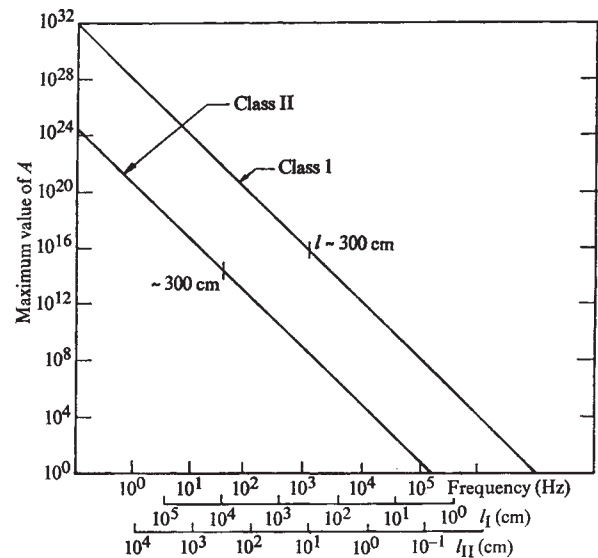


Fig. 1 Maximum power deliverable by a gravitational detector to a recording device is proportional to A , where $A = 2\pi f_0 M l^2$.

ends. The frequency of vibration of a beam of length l and thickness a is⁴

$$f_0 = \frac{\pi v_s a}{2\sqrt{12} l_2^2} \quad (11)$$

It does not depend on the width b . Equation (11) is strictly valid only if $\eta_2 < 1$, where $\eta_2 = a/l_2$. The mass is

$$M \approx \rho 4ab l_2 \quad (12)$$

Also, for practical reasons $\beta_2 < 1$, where $\beta_2 = b/l_2$. In terms of these quantities

$$A_{11} = (\pi^6 3^{-5/2} \rho v_s^5 \eta_2^6 \beta_2) / (128 f_0^4) \quad (13)$$

Choosing $\eta_2 = \beta_2 \sim 1/15$ as reasonable values and taking for v_s and ρ the same values as for the class 1 calculation, we obtain the curve given in the figure for class 2 detectors. The class 2 curve is similar to the class 1 curve, but is moved to lower frequencies by approximately two decades. Applying the same arbitrary criterion that detectors larger than 300 cm are impractical, we are restricted to frequencies larger than 30 Hz, and values of A less than 10^{15} . This value of A is comparable with that of the Weber detector. Thus, in a frequency region where class 1 detectors may be too expensive, class 2 detectors are feasible (according to the same criterion by which class 1 detectors are judged) and can have power sensitivities comparable with those of class 1 detectors in their frequency range. It is extremely important for astrophysical and cosmological reasons to attempt to observe gravitational radiation at frequencies different from 1,660 Hz (the frequency of Weber's detector). The resulting observations of the power spectrum would be valuable in determining the nature of the sources.

It should be pointed out that class 2 detectors can distinguish between scalar and tensor radiation in a way that class 1 detectors cannot (without difficulty). The quadrupole vibrational mode (or fundamental) of the "hollow" square will not be excited by scalar radiation; conversely the next highest mode will be excited by scalar radiation but not by quadrupole radiation. Thus in the same apparatus there is a "pure quadrupole" channel and a "pure scalar" channel, whereas in class 1 detectors the modes can be excited by either quadrupole or scalar radiation. Moreover the "hollow" square antenna is sensitive to only one state of tensor polarization, so that by rotation, polarization studies of the sources can be made.

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assumption of frequency dependence is unnecessary and unjustified".

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Drs Woodward and Yourgrau write :

DR REINHARDT is correct in saying that the time delay and quasar deflexion results flatly contradict the frequency dependence hypothesis presented in our first paper¹. We do not take exception to the reported results that Dr Reinhardt quotes. We only note below some interesting features of those results. We would like to mention that it is possible, following a suggestion of Treder², to formulate a plausible theory of the electromagnetic-gravitational field interaction that easily explains all of the empirical data, including internal anomalies in the observations that indicate frequency dependence. A paper describing this theory is now in preparation.

Though there is a difference by a factor of three in the density of the r^{-6} component of the plasma density if one demands the same deflexion for both Seielstad's and Muhleman's observations^{3,4}, we do not feel that the reported results of the quasar deflexion experiments are significantly in error.

In the results of the interplanetary time delay experiments, we accept the general validity of the stated conclusions, but we should like to point out two anomalies that would be expected if some frequency dependence, albeit far smaller than would be expected on the basis of our first paper¹, were operative. Orbits determined using radar data are somewhat spurious, and prediction and observation at later epochs disagree. According to Ash *et al.*⁵, "... the predictions based on our fitted orbits were compared with Earth-Venus and Earth-Mercury time delay measurements obtained from about one month to six weeks after the last datum included in the analysis. The differences (O-C) ranged from 50 to 80 μ s. No completely satisfactory explanation has been found for these discrepancies; less than one quarter can be accounted for solely by errors in the parameter estimates of magnitudes comparable to the standard errors of those estimates". A plasma hypothesis, tentatively advanced by the authors as an explanation, was conclusively ruled out by Smith *et al.*⁶.

Another anomaly is that simultaneous ranging at different frequencies, at least in some circumstances, gives different time delays. In fact, "... a programme undertaken in the spring and summer of 1967 to make simultaneous interplanetary time delay measurements at Cornell's Arecibo Ionospheric Observatory ($\lambda=70$ cm) and Haystack disclosed slowly varying, systematic differences in the delays—about 20 μ s on average. These differences disappeared at the close approaches between the Earth and the target planet but increased gradually as the interplanetary distance increased. No satisfactory explanation for these discrepancies has yet been found; neither plausible errors in timing nor effects of interplanetary plasma can account for them⁷". Even if the above cited anomalies are totally physically insignificant, it is still possible within the formalism of the theory now in preparation to account for null results at the frequencies used in the time delay and quasar

A Paradox in the Interaction of the Gravitational and Electromagnetic Fields ?

RECENTLY Woodward and Yourgrau¹ put forward the hypothesis that the influence of gravitation on the propagation of electromagnetic waves depends on the frequency of the radiation. Their starting point is that the light deflexion by the Sun's gravitational field, as found from optical determinations at solar eclipses, appears somewhat larger^{2,3} than the value predicted by Einstein's theory of gravitation. Lately Muhleman *et al.*⁴ and Seielstad *et al.*⁵ succeeded in measuring the light deflexion by the Sun at 2.388 GHz and 9.602 GHz. Their results ($1.82'' \pm 0.2''$ and $1.77'' \pm 0.2''$) are in close agreement with the predictions of general relativity. The uncertainty introduced by our limited knowledge of the coronal plasma amounts to about 10 per cent of the relativistic light deflexion⁴, which, being sufficient to prevent a decision between Einstein's theory and the Brans-Dicke theory, is not enough to make these measurements compatible with the predictions of Woodward and Yourgrau. Their hypothesis (according to formula (2), or (1) and (3))¹ gives at 2.388 GHz and 9.602 GHz the tremendous values of $15' 39''$ and $7' 45''$ respectively for the light deflexion (that is, more than 500 times and 200 times the experimental results).

This is not astonishing, as Woodward and Yourgrau¹ derived the coefficients determining the wavelength dependence of the light deflexion chiefly from the experiments of Sadeh *et al.*^{6,7}. Sadeh *et al.*⁶ found a frequency shift of the 21 cm line in Tau A near an occultation by the Sun, which was orders of magnitude higher than the general relativistic value. Yet such an anomalous frequency shift (or time delay) was not confirmed, either by the radar measurements of Shapiro and co-workers^{8,9} or by the recent Mariner VI and VII results¹⁰, all of which yielded the general relativistic values with an uncertainty of about 10 per cent^{8,9} or better¹⁰. Thus the suspicion arises that there was an unknown source of error in the experiment of Sadeh *et al.*⁶. The clock experiment of Sadeh and coworkers⁷ has not yet been repeated (to my knowledge) and one should await further measurements before drawing far reaching conclusions.

It seems that the recent experiments^{4,5,8-10} preclude the possibility of a frequency dependence of the interaction of gravitational and electromagnetic fields, at least at the strength assumed by Woodward and Yourgrau. To cite the authors¹ (and replacing an "if" by an "as"): As the "observed deflexion corresponds to that predicted by the general theory . . . the

deflexion experiments, while still explaining the observations of Sadeh, Ball⁸ and others.

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Mechanism for the Fixation of Nitrogen by Lightning

THE results of present studies directed towards the solution of the ion chemistry of the ionospheric D-region have interesting implications for the lower atmosphere as well. A major ion product of air ionization is NO⁺, which is a "terminal" ion because of its low energy content. Reactions which produce NO⁺ are¹



These reactions cause NO⁺ to be a dominant ion in the regions of the Earth's ionosphere where neutral NO is a minor constituent. The reaction

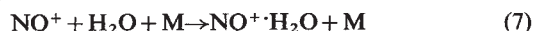


is exothermic, and while known to be slow¹, may still be of significance where N₂ densities are very high. The N₂⁺ produced in air rapidly produces O₂⁺ by charge-transfer

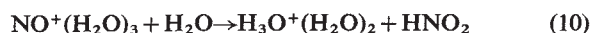


Finally, it is known^{2,3} that all of the atmospheric ions, N⁺, N₂⁺, O⁺, O₂⁺ and NO₂⁺, charge transfer rapidly to NO to produce NO⁺. Thus a large fraction of the positive ions produced in an atmospheric ionization event, such as a lightning stroke, can be expected to lead to NO⁺ production.

Recently it has been shown^{4,5} that in a wet atmosphere NO⁺ will hydrate with three waters of hydration, that is



Following the formation of NO⁺(H₂O)₃, a fast binary reaction occurs



These reactions are all known to be efficient: $k_7 = 1.5 \times 10^{-28}$ cm⁶ molecule⁻² s⁻¹ (ref. 5); k_8 and k_9 are roughly two and three times faster and $k_{10} \approx 10^{-9}$ cm³ mol⁻¹ s⁻¹.

The production of ionization in a wet atmosphere, therefore, leads with high efficiency to the fixation of nitrogen in the form of nitrous acid which, of course, may then react further.

The above reaction scheme and an analogous scheme starting with O₂⁺ are responsible for the dominance of water cluster ions, H₃O⁺(H₂O)_n, in the lower ionosphere of the Earth; that is, at altitudes below about 80 km^{4,6}. The production of HNO₂ has been verified by Puckett and Lineberger in negative ion studies of NO-H₂O afterglows⁷. The production of nitrous acid has been observed in irradiated air following nuclear tests⁸.

There seems to be little reason to doubt that the above reaction sequence will occur following lightning bursts (and other ionizing events) in the Earth's lower atmosphere with a high efficiency of nitrogen fixation; that is, a high ratio of nitrous acid produced to ion pairs produced.

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Carbon Dioxide in the Surface Water of the Ice-covered Bering Sea

DETERMINATION of the equilibrium concentration of CO₂ in the ocean surface with respect to air is necessary in order to understand the interaction of CO₂ between the atmosphere and the ocean. The concentration of CO₂ in water depends on its physical, chemical and biological properties. The oceans, therefore, act as both sources and sinks for CO₂ in the air. Keeling¹ reported that high concentrations of CO₂ in the ocean appeared near the equator and were lower in the sub-tropics of all oceans. Areas along the west coasts of continents where upwelling occurs have high CO₂ concentrations. In the polar regions, patterns are more complicated primarily because of a lack of seasonal data. Measurements of the equilibrium concentration of CO₂ with respect to air in the surface waters of the Kara and Barents Seas during the late summer showed that the surface waters were supersaturated with CO₂ near the mouths of the Ob and Yenisey Rivers and undersaturated in the Barents Sea². The present study was undertaken to measure the actual distribution of CO₂ between the atmosphere and the sea over open leads and polynyi in the ice-covered Bering Sea. Previous measurements of CO₂ in ice-covered seas were made by Kelley³ in the water under the Arctic Ocean pack ice near Ice Island T-3.

A recent cruise of the US Coast Guard ice breaker Northwind during February 1970 provided an opportunity to visit

the region around the southern limit of the winter sea ice pack in the Bering Sea. The ice breaker was able to search out and select areas of open water in the constantly shifting pack. Oceanographic stations were established (Fig. 1) after the ship manoeuvred into a lead or polynya and shut down its engines and overboard waste disposal to reduce contamination of the surface water. The line showing the southern limit of the ice (Fig. 1) is based on the best information to be had at the time from ice reconnaissance reports from aircraft. Several days before sampling, open water prevailed at Stations A and B (Fig. 1). At the time that the observations were made, the ice had drifted south beyond the stations. The ice thickness at all the sampling sites was less than 50 cm.

Measurements were made from a small mobile laboratory (7×8×12 feet) secured to the starboard deck of the ship. Water was pumped by means of a submersible pump to a sea chest located on the deck (Fig. 2). The equilibrator consisted of a plastic canopy floating on the surface of the water in the sea chest. Air was continuously circulated in a closed loop containing a diaphragm pump, flow gauge, moisture trap, glass bubbler and equilibrator. An automatically operated solenoid valve system was provided to shunt the air into a second circuit which included the infrared analyser, MSA LIRA 200, without

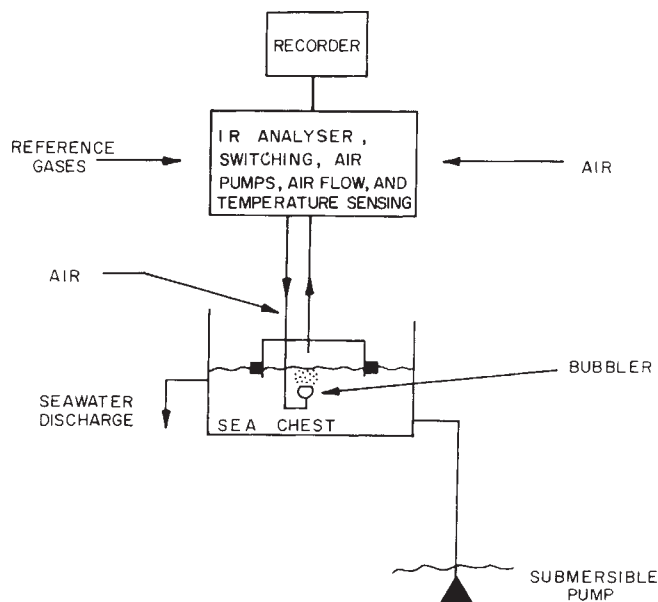


Fig. 2 Schematic diagram of the analytical system for measuring CO_2 in seawater.

interrupting the flow and with no loss of gas. The temperature of the water in the sea chest was monitored continuously. The temperature was compared with temperatures recorded from the reversing thermometers attached to Nansen bottles on the hydrographic wire located at the same depth as the submersible pump. In all cases the differences in temperature between the actual sea surface and sea chest temperatures were less than 0.1°C . Air was drawn through 'Tygon' tubing from a point near the bow of the ship and ahead of the ship's stack about 10 m above the surface of the sea. Measurements of CO_2 in the air, after equilibration with seawater, and in reference gases were made every half hour.

The concentrations (mixing ratio) of CO_2 are reported in parts per million by volume dry air. Positive departures of the concentrations of seawater CO_2 from equilibrium with the atmosphere are referred to as conditions of supersaturation.

The equilibrium concentration of CO_2 in the seawater restricted by leads and polynya in an ice covered sea showed that the water was supersaturated in CO_2 with respect to air (Table 1) and the magnitude of the supersaturation was relatively constant over a wide area of the sea ice pack of the south-eastern Bering Sea. The average concentration difference between CO_2 in the sea and air during the cruise of the Northwind was closely comparable with that observed in this particular region of the Bering Sea during the summer when ice free conditions prevailed (Table 2)⁴. Water under the Arctic ice near Ice Island T-3 (ref. 3) also showed CO_2 supersaturation with respect to air, higher than the observed concentrations of CO_2 in the open leads and polynya.

Open water in ice-covered areas during the winter may be important in assessing the atmospheric inventory of CO_2 in the Arctic. Unlike the summer, when autotrophic plant

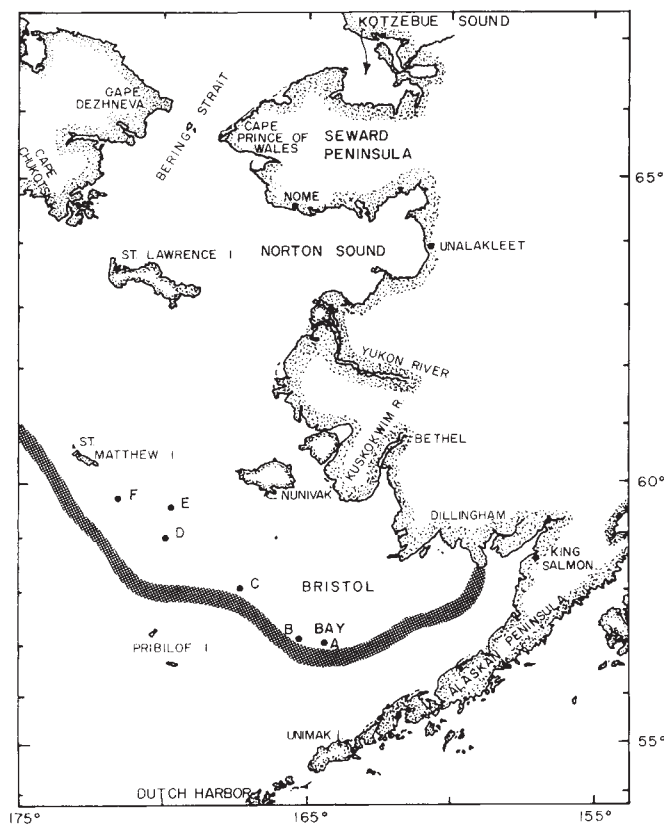


Fig. 1 Map of the south-eastern Bering Sea showing the southern limit of the sea ice during February 1970 and the location of the oceanographic stations.

Table 1 Concentration of CO_2 in the Air and Seawater: February 1970

Station	Date	Latitude (degrees N)	Longitude (degrees W)	Carbon dioxide Air (p.p.m.)	Carbon dioxide Sea	Air temperature ($^\circ\text{C}$)	Wind speed (knots)	Water depth (m)	Seawater temperature ($^\circ\text{C}$)	Salinity (parts per thousand)
A	3	57° 5'	164° 22'	325.2	343.9	-14.7	14	69	-1.71	31.826
B	4	57° 7'	165° 15'	324.7	338.1	-15.0	14	63	-1.66	31.905
C	4	58° 14'	167° 26'	324.5	336.3	-13.8	13	63	-1.71	31.848
D	5	59° 5'	169° 58'	324.8	334.1	-5.9	22	63	-1.64	31.863
E	5	59° 31'	169° 53'	324.5	342.1	-6.0	17	54	-1.66	31.685
F	6	59° 45'	171° 22'	325.6	337.5	-5.5	4	99	-1.67	32.059

Average CO_2 concentration, air = 324.9; average CO_2 concentration, sea = 338.7.

Table 2 Carbon Dioxide Concentrations in the Bering Sea and Arctic Ocean

Location	Date	Average concentration difference of CO ₂ between sea and air	Sea surface conditions
Bering Sea (this article)	February 1970	13.8	Ice covered sea
Arctic Ocean, 78°N, 174° W	January 1967	41.9	Ice covered sea
Bering Sea, 57° to 60° N, 168° W	July 1968	15.5	Ice free
Bering Sea, 57° to 60° N, 168° W	September 1968	7.0	Ice free

blooms on land and sea develop and provide a sink for CO₂, the northern Arctic and subarctic regions are covered with snow and ice for several months of the year and provide no biological sink for CO₂.

The importance of open water in air-sea interactions, which may result in the transfer of CO₂ from the sea to the lower atmosphere, is emphasized by Whitman and Schule⁵, who estimate that the yearly variation of open water during the winter may be as much as 11% in the Eurasian Basin and 12% in the Canadian Basin. It has already been shown by Badgley⁶ that turbulent heat flux to the atmosphere increased about a hundred-fold during the winter over an artificial open lead.

The data for CO₂ in the south-eastern Bering Sea suggest that seawater is a potential source of CO₂ in this area, as in the northern part of the Eurasian Basin of the Arctic Ocean³. If the ice were removed for a significant period of time, as in leads and polyny, then CO₂ could be released from the seawater to the atmospheric reservoir.

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Magnetic Field Structure around the Crab Pulsar

LINEAR polarization observations of the Crab nebula at optical wavelengths indicate that the magnetic field is uniform in direction over a large region (about 0.5 pc) around the pulsar. Observations were made with a completely automatic sky compensated polarimeter-cum-photometer with an S20 photomultiplier and a GG13 filter. A 'Fabri-Tek' computer in the pulse counting mode was used to store data for each degree of rotation of the polaroid filter during each observation and a digital magnetic tape recorder was used for permanent

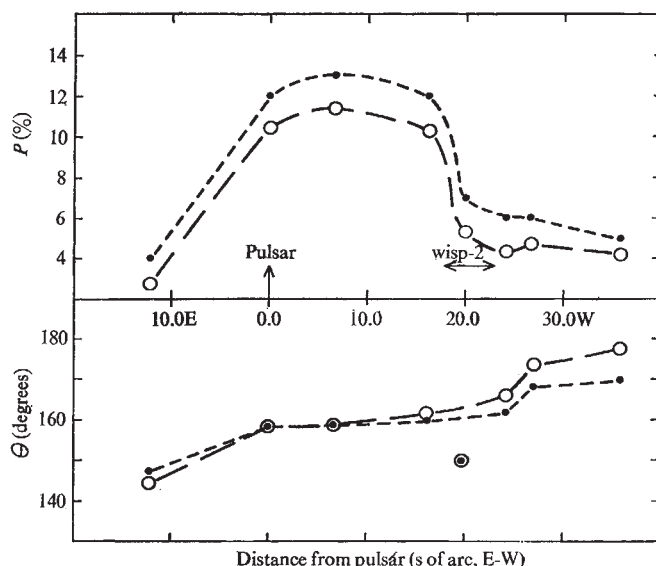


Fig. 1 P and θ of eight patches around the pulsar are plotted against the distance of these patches from the pulsar. ●, Not corrected for interstellar polarization; ○, corrected for interstellar polarization.

data storage. A 'CDC 6400' computer was then used for direct reduction of the magnetic tapes. A complete description of the instrument and data system will be given in a later publication. Observations were made on November 4 and 5, 1969, and on February 1 and 2, 1970, with the 84 inch telescope at Kitt Peak. Three standard stars, HI 29, HDE 253683 and HDE 251204 (ref. 1), were used to calibrate the observations and an unpolarized star was used to evaluate the instrumental polarization. All angles reported in this paper are measured from north towards east.

The observations were made predominantly along a line about 55 arc seconds (east-west) centred about 12 arc seconds to the west of the pulsar. These are part of a programme to study the magnetic field in the region around the pulsar and to clarify the relationship between the field and the wisps^{2,3}.

Table 1 Results of Polarization Observations with Different Size Apertures Centred on the Crab Pulsar

Date of observation	Aperture (arc seconds)	Angle θ°		Polarization P (%)	
		Corrected for interstellar polarization*	Uncorrected	Corrected for interstellar polarization*	Uncorrected
November 4, 1969	20	157	156	10.8	12.4
November 4, 1969	10	157	156	10.9	12.5
November 4, 1969	8.7	159	158	10.9	12.5
November 4, 1969	8.7	161	159	11.0	11.6
February 2, 1970	2	162 ± 6	160	11.5 ± 2.5	13.1

* To correct for interstellar polarization, HD 36879 (the star nearest the Crab nebula in Hiltner's catalogue⁸) was used. Because the correct value in the direction of the nebula itself is uncertain, both corrected and uncorrected values are given.

The results of five observations centred on the pulsar, with apertures of four different sizes, are given in Table 1. Although the pulsar was included in these observations, the values of the polarization P and the position angle θ of the electric vector in the table have been corrected for the polarization of the main and subpulses, which is 6.8% at 98° (see ref. 4). In the case of the observation with the 2 arc seconds aperture the light of the pulsar was included in the aperture for only part

Table 2 Results of Polarization Observations of the Crab Nebula

Num- ber	Position relative to pulsar (arc second)		Date of observation	Angle θ°		Polarization P (%)		Aperture diameter (arc second)
	E-W	N-S		Corrected for interstellar polarization *	Uncor- rected	Corrected for interstellar polarization *	Uncor- rected	
1	12.1 E	—	Feb. 2, 1970	144 ± 8	147	2.6 ± 1.2	4.2	2
2	Pulsar	—	Nov. 4, 1969	159 ± 1	158	10.5 ± 0.5	12.1	8.7
3		—	Nov. 4, 1969	159 ± 1	158	11.4 ± 0.5	13.0	8.7
4		—	Nov. 4, 1969	162 ± 1	160	10.4 ± 0.5	12.0	8.7
5	20.1 W	—	Nov. 4, 1969	150 ± 3	150	5.4 ± 0.5	7.0	8.7
6	24.2 W	—	Feb. 2, 1970	166 ± 3	162	4.4 ± 0.5	5.9	2
7	26.8 W	—	Nov. 4, 1969	174 ± 3	168	4.7 ± 0.5	6.0	2
8	36.3 W	—	Feb. 2, 1970	177 ± 3	170	4.2 ± 0.6	5.4	2
9	19.0 W	19.0 S	Nov. 4, 1969	162 ± 2	160	8.4 ± 0.5	10.0	8.7

* See Table 1.

of the integrated time. Thus it was necessary to derive the 2 arc seconds aperture results indirectly, by using magnitudes of 16.5 for the pulses⁴ and 18.6 per square second for the nebula. The magnitude of the nebula is derived from observations made with an 8.7 arc seconds aperture.

Table 2 shows the results of nine observations, eight of them along the east-west axis through the pulsar. Fig. 1 shows plots of P and θ versus the position of apertures along the east-west axis. Using Fig. 1 one can see a region of constant P and constant θ , asymmetrically situated, centred about 8 arc seconds to the west of the pulsar and extending about 8 arc seconds to each side. These observations along with those in Table 1 clearly indicate that the magnetic field structure is very uniform in the region mentioned above, and the fine scale structure is smaller than 0.02 pc. On the assumption that the radiation of the nebula is of synchrotron origin, the uniform field in this region is at an angle of 68° . It is interesting to note that the elongation of this region and the wisp activity both occur in the half plane to the west of the pulsar.

Fig. 1 also shows that the angle of polarization slowly and smoothly increases from east to west, with one exception. This exception, observation 5 (Table 2), lies off the curve and

differs by about 15° from the two neighbouring observations. Using the reproductions of Scargle and Harlan⁵ we find that this observation lies on the edge of wisp 2 (at epoch d , which is November 10, 1969). Furthermore, we measure the position angle of the axis of the wisp from the reproductions as approximately 40° . This differs by about 28° from the direction of the general magnetic field in this region. Scargle and Harlan⁵ suggest that these wisps are plasma waves generated by relativistic particles from the pulsar. If this is the case, we can calculate ζ , the angle through which the magnetic field is rotated by the passage of a suprathermal hydromagnetic wave. Following Parker⁶, we have for the hydromagnetic equation, assuming small amplitudes

$$\frac{\partial \mathbf{b}}{\partial t} = \mathbf{B} \nabla \times (\mathbf{v} \times \hat{\mathbf{e}}_z) \quad (1)$$

where $\mathbf{b}$ is the magnetic perturbation, $\mathbf{v}$ is the velocity perturbation of the suprathermal material; we have assumed a uniform magnetic field $\mathbf{B}\hat{\mathbf{e}}_z$. Assuming, as Parker does, a plane wave with wave vector $\mathbf{k}$ and frequency ω and taking $\mathbf{k}$ in the yz plane and ψ the angle between $\mathbf{k}$ and the z axis (direction of the magnetic field), we find that the plane wave takes the form

$$\exp i[\omega t - k(z \cos \psi + y \sin \psi)] \quad (2)$$

which yields for equation (1)

$$b_x = 0 \quad b_y = -\frac{B v_y \cos \psi}{\omega/k} \quad b_z = \frac{B v_y \sin \psi}{\omega/k}$$

Taking $\psi \sim 62^\circ$ (angle between $\mathbf{k}$ and $\mathbf{B}$) and assuming $v_y =$ sound velocity $= c/\sqrt{3}$, we find $\zeta = 20^\circ$ if $\omega/k = 0.3$ (ref. 5) or $\zeta = 25^\circ$ if $\omega/k = 0.2$ (ref. 7). The relevant observation (No. 5) gives a result of this order. More interesting is the agreement of this rotation of 20° – 25° with the rotation of the axis of the wisp about 28° away from the direction of the general field. Thus although the general field around the pulsar is at an angle of 68° , the field in the wisps may lie along their axes, as Scargle³ claims, and this rotation of about 28° may be caused by the propagation of suprathermal waves through the nebula.

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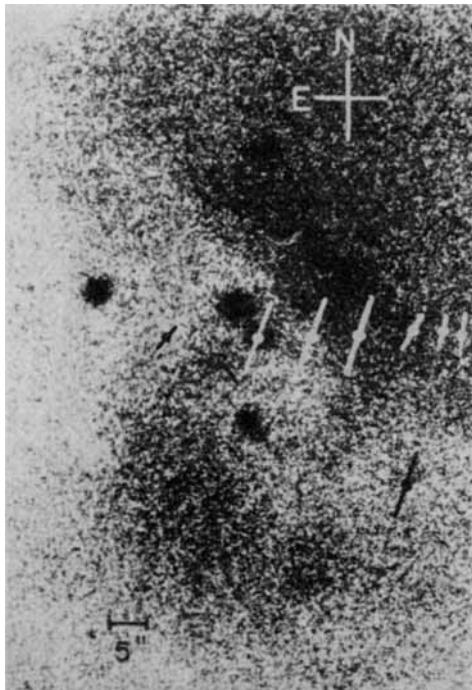


Fig. 2 A reproduction of Scargle and Harlan's plate of the Crab nebula (ref. 5) taken on November 10, 1969 (epoch d). All the patches (except number 8) for which polarization measurements were made are marked and their positions are listed in Table 2. The length of the bar at each point is proportional to the polarization observed and the orientation is that of the electric vector.

Kinetic Mass Spectrometric Study of the Flash Photolysis of NO₂

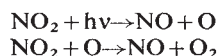
WE have re-examined the flash photolysis of NO₂ using a flash photolysis system coupled to an EAI 'Quad 200' series mass spectrometer. Initial experiments indicated that efficient electrical and optical screening must be provided between the flash lamp and the mass spectrometer chamber. A reactor with an aluminium outer case and base-plates was used to eliminate interference by the flash with the operation of the spectrometer and with the size of the background peaks m/e 17, 18, 28 and 44 resulting from desorption from the chamber walls. The nipple which protruded into the ion source was fitted with a molybdenum disk with a 50 μ m aperture supplied by Aeon Laboratories. We calculated that in this way more than 80% of the reactants passed through the aperture directly into the ionization chamber and the spectra obtained were representative at all times of the composition of the gases inside the reactor. The outside wall of the reactor consisted of a glass tube 2.5 cm in diameter, 10 cm long, carrying a B24 conical socket joint and fastened with 'Araldite' into the base plate. A quartz flash lamp filled with 15 Torr of argon fitted coaxially inside the reactor. A 0.001 μ F capacitor was connected across the leads to the flash lamp to eliminate interference from silent discharge during the charging of the main capacitor. The half-width duration of the flash was about 40 μ s. The mass spectrometer signal was either displayed on an oscilloscope and photographed or recorded by an ultraviolet oscillograph.

The technique was checked by flash photolysing a 3 : 1 mixture of hydrogen and nitrogen dioxide. When profiles of peaks at m/e 17 and 18 were compared, it was found that there was an initial excess intensity of the m/e 17 peak which corresponded to that attributed by Meyer, in a similar experiment¹, to hydroxyl radicals.

The technique was found to be suitable for monitoring single ion peaks with 10 μ s resolution or for providing complete mass spectra every 10 ms. Linear response of the system to reactant concentrations was achieved by working with reactor pressures which gave molecular flow through the 50 μ m aperture. This made the addition of inert gas at high pressures impossible so experiments could not be conducted under strictly isothermal conditions.

Preliminary experiments have been performed on the flash photolysis of NO₂ at pressures of 0.33 to 1.48 Torr in the presence of 0.13 Torr of argon and in one case of 0.31 Torr of SF₆. A typical set of profiles, normalized against that of the argon m/e 40 peak, shown as a broken line, is illustrated in Fig. 1. Changing the pressure of nitrogen dioxide and adding SF₆ had little effect on the shapes of these profiles.

The peak m/e 32 can be attributed entirely to oxygen, because none of the oxides of nitrogen gave this peak and there was no ozone peak at m/e 48. The overall increase in oxygen and changes at m/e 30 and 46 cannot be explained by any of the mechanisms postulated by previous workers². The sharp initial changes in these peaks arise chiefly from



but these reactions are too fast to account for the slower changes which occur up to 300 ms later. These changes cannot be explained by the thermal decomposition of residual nitrogen dioxide, because a temperature of about 1,300 K would be necessary³ and this seems most likely 100 ms after flash. Also, the rate of oxygen formation and the decrease at m/e 46 were both linearly dependent on the pressure of NO₂, whereas the thermal decomposition follows a second order function⁴. Huffman and Davidson⁵ have shown that the unimolecular decomposition of NO₂ only becomes important at temperatures of about 3,000 K. The increase at m/e 46 after 40 ms cannot be due to the third order reaction of NO and O₂ which is 10 times too slow⁶. Comparison of the profiles reveals an initial mass

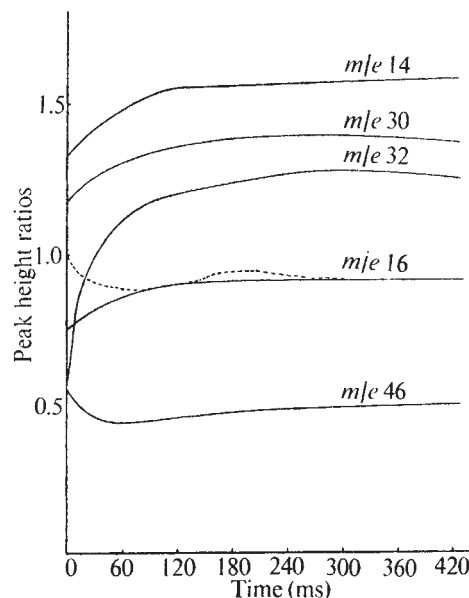


Fig. 1 Peak profiles for flashed NO₂. NO₂ 0.69 Torr, argon 0.13 Torr. Profiles m/e 14, 16, 30 and 46 plotted as a ratio of their pre-flash peak heights. Profile m/e 32 plotted as a ratio of the peak height produced by 0.138 Torr oxygen.

discrepancy which decays in parallel with the changes at m/e 30, 32 and 46, indicating a reaction involving some species other than NO₂, NO and O₂, either in the gas phase or on the walls. Similar profiles for these peaks were obtained in vessels constructed entirely of quartz so that the reactions cannot be attributed to the presence of aluminium, molybdenum or 'Araldite'.

A search for peaks at other mass numbers proved fruitless, but this does not rule out the presence of other oxides of nitrogen, for N₂O₅ gave the mass spectrum m/e 46 (72%), 30 (100%), 16 (10%) and 14 (4.5%). Similar spectra were obtained irrespective of whether the N₂O₅ was admitted through the reactor or through an all glass capillary inlet. Of the oxides of nitrogen only NO₃ and N₂O₅ are likely to produce oxygen in these conditions, and Husain and Norrish⁷ have shown that gaseous NO₃ has a life time of 40 μ s and therefore cannot account for the observed reactions. The discrepancy in the mass balance cannot be explained by the presence of gaseous N₂O₅, which in any case only produces NO₂ in the presence of O (ref. 8). We therefore conclude that the flash photolysis of NO₂, an intermediate is rapidly formed, probably on the surface, which then reacts more slowly to produce O₂ and NO₂.

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Nitrate and Ammonium Contents of Wisconsin Limestones

It has been suggested^{1,2} that a number of environmental problems may be associated with high levels of nitrates and other nitrogenous compounds in waters. These problems relate to deleterious effects on human and animal health, crop quality and yields, and eutrophication of surface waters. Nitrate in the environment may be derived from nitrogenous fertilizers, organic wastes, soil organic matter, precipitation, and biologically fixed nitrogen. According to Feth³, however, geological sources of nitrate have been largely ignored, and he has suggested that high levels of nitrate in water may be associated with limestones. We therefore measured the nitrate content of Wisconsin limestones.

Nitrate-nitrogen was determined in 210 limestone samples from quarries throughout Wisconsin. Major geological formations sampled were Niagara (Silurian), Galena Black River (Ordovician), Lower Magnesian (Ordovician), and Mendota (Cambrian) dolomites. Determinations were made with the nitrate-selective ion electrode following the procedure of Bremner, Bundy, and Agarwal⁴, except that a 10 g sample (80-mesh) and 50 ml. of 0.01 M calcium sulphate were used. Nitrate-nitrogen and ammonium-nitrogen were also determined in seventy-four of these samples using the direct steam-distillation method of Keeney and Bremner⁵. Close agreement was found between nitrate-nitrogen values determined by the two procedures.

All samples contained measurable amounts of these forms of nitrogen, and values as high as 37 p.p.m. nitrate-nitrogen and 18 p.p.m. ammonium-nitrogen were obtained (Table 1). There seemed to be no relationship between nitrate content and type of limestone.

Table 1 Distribution of Nitrate—N⁴ and Ammonium—N⁵ in Limestone Samples

N content (p.p.m.)	Number of samples	
	NO ₃ -N	NH ₄ -N
<2.5	135	49
2.6–5.0	48	15
5.1–7.5	8	6
7.6–10.0	9	3
10.1–15.0	5	0
15.1–20.0	3	1
20.1–25.0	1	0
>25.1	1	0
Total	210	74

Although ammonium nitrate is often used to blast limestone (about 1 kg per 5,000 kg rock), it undergoes decomposition to form nitrogen gas, nitrogen oxides and water, and contamination of samples by ammonium nitrate therefore seems unlikely. This was confirmed by analysing samples from a quarry adjacent to two recent blasts and from an area not blasted for 10 years. There was no difference in the nitrate or ammonium content of these samples (all less than 2 p.p.m. nitrogen), indicating that these forms were occluded within the rock.

Investigations involving the effect of grinding or of dissolving limestone in HCl showed that the nitrate and ammonium in limestones were present entirely as soluble salts. These forms of nitrogen are therefore retained by physical entrapment, and are released eventually on the weathering of the rock.

Although the nitrate and ammonium values obtained for a large number of samples seems relatively low (two-thirds contained less than 2.5 p.p.m. of each form), consideration of the amount of nitrogen in a layer of rock gives a different picture. For example, assuming an average density of 2.5 g per cm³ for limestone, 1 p.p.m. of nitrogen is equivalent to 25 kg of nitrogen per hectare-metre. Several samples contained at least ten times this amount of nitrate-nitrogen.

Our results indicate that many limestones are a potential source of nitrate to percolate waters and that geological

contributions of nitrate should be considered when evaluating sources of nitrate to ground waters.

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Subglacial Limestone Deposits in the Canadian Rocky Mountains

FORD *et al.*¹ have recently reported the discovery in the Rocky Mountains of Canada of calcite deposits which they have ascribed to a subglacial origin. The occurrence of such subglacial limestone deposits is, however, neither as rare in glaciated lands nor as neglected in the literature as they seem to believe. The problems of genesis noted by Ford *et al.* are therefore reviewed here in the context of a wider literature.

The first detailed discussion of deposits which are now considered to be subglacial limestone is probably that of Höeg², who supposed them to be postglacial marine deposits. The latest and most comprehensive study of subglacial limestone is that of Kers³. In addition to a historical review of the literature, Kers describes deposits of subglacial limestone from several new localities in Scandinavia. He concludes that the limestones were formed by the solutinal and depositional agency of pressure meltwater at the ice sole; a conclusion which essentially follows a theory first advanced in 1936 by Ljungner⁴.

In the account of Ford *et al.*, however, there seems to be an implicit assumption that the subglacial limestone deposits of the Mt Castleguard area were derived from free subglacial water. The agency of pressure meltwater is not considered. Yet pressure meltwater would seem to resolve the problems of genesis reported by Ford *et al.* The authors are puzzled because solute carbonate concentrations were found to be only small in the free meltwater streams of the Mt Castleguard area. But in the context of a pressure meltwater hypothesis, the chemical composition of these free meltwaters is not relevant. Similarly, the authors' assertion that "the environment" cannot be notably enriched in carbon dioxide is misplaced. The transposition of such "calcite precipitates" must require an agency rich in carbon dioxide and, indeed, there are strong grounds for supposing that the ice and pressure meltwater of the glacier sole would fulfil this requirement.

Corbel⁵ has stressed the high concentration of carbon dioxide both in snow and snow-derived meltwater, and Williams⁶ has shown that the carbon dioxide content of air within a snowbank may reach a concentration twice that in free air. Williams attributes this concentration of carbon dioxide to daily freezing and thawing at the surface of the snow. Freezing of meltwater releases the carbon dioxide it has absorbed from the atmosphere. Because it is a heavy gas, the carbon dioxide remains in the snowbank and rain or meltwater passing through can absorb the gas to the point of saturation. In this way the water is provided with carbonic acid for chemical weathering.

BIOLOGICAL SCIENCES

Flandrian History of the Teesdale Rarities

THE survival of the Teesdale rarities through the Flandrian period¹, during the prominence of inimical conditions that led to the development of woodland and blanket peat and the podsolization of the soil, suggests either that the present habitats have provided similar conditions since the widespread distribution of the rarities in Late Glacial times² or that the habitats are of recent origin and the plants survived elsewhere³. Neither of these hypotheses is based on the ecological history of Upper Teesdale but on a comparison of the present flora with that of Late Glacial times in other areas and on the assumption that the vegetational history of Upper Teesdale has followed a similar course to that of other floristically rich areas (for example, Ben Lawers⁴, Cwm Idwal⁵).

Palaeoecological evidence is now available for the vegetational history of Cronkley Fell in the North Riding of Yorkshire where many of the rarities are found⁶. Organic deposits started to form in persistently waterlogged habitats (for example, surface depression and hillside structural benches⁷) where *Phragmites communis* became prevalent in Zone V⁸. The earliest record of woodland indicates that in Late Boreal times (Zone VI⁸) unstable conditions—fluctuating water levels and increasing temperatures—greatly influenced the vegetation which, by the end of the period, was composed of the following: extensive ?(oak)–birch–hazel scrub, probably to the limit of tree growth⁹; restricted localities with oak, elm, pine and alder, whose distribution was affected by soil conditions; and hollows containing reedswamp. The successive establishment of hazel, oak, elm and pine during this time seems to have been largely dependent on edaphic factors and thus oak and elm became established before pine as they seem to have done in parts of the Tees Valley (for example, Romalldkirk⁹).

The expansion of alder at the start of the Atlantic period has been attributed to an increased precipitation/evaporation ratio which caused, or aided in, the degradation of Boreal vegetation¹⁰ and helped in the initiation of blanket peat. Pollen spectra reveal the following Atlantic vegetation: restricted areas of oak and elm around the drift free limestone outcrops—more or less unaffected by blanket peat growth; damp peaty hollows with alder, birch and hazel on the margins; patches of the once extensive ?(oak)–birch–hazel scrub—most affected by blanket peat growth; expanding areas of blanket peat; areas of instability (for example, springheads, screes) which were devoid of vegetation or had a poor patchy ground flora of grasses, sedges and rushes—initially free from blanket peat.

It is impossible to delimit a tree line (that is, a line above which no trees occurred) during VIIa⁸, even if pollen analysis was sensitive enough to do so, principally because edaphic rather than climatic factors seem to have played a major part in determining the vegetation disposition. The limit of closed woodland may be placed, however, around 365 m, although many extensive patches of oak scrubland, which may have been similar in composition to the contemporary high level Pennine and Lake District oakwoods^{11,12} and localized patches of oak and elm, existed at higher altitudes.

As much of the land above 365 m carried at least a scrubland or was devoid of vegetation, a change in the water economy was necessary for the initiation of blanket peat during the Atlantic¹³. This change may have been caused by the increased precipitation/evaporation ratio but numerous charcoal fragments throughout the peat suggest some biotic factor was significant. The chief factor in the destruction of the Atlantic vegetation may therefore have been anthropogenic interference¹⁴. Although peat may have been the natural outcome of increased rainfall on poorer soils¹⁵, it might be questioned whether, without human intervention, the areas adjacent to the lime-

Compaction, melting and refreezing in the firn regions of glaciers must magnify this snowbank process and lead to the entrapment of air pockets rich in carbon dioxide, to be retained within the ice of the glacier even down to the snout^{7,8}. Coachman *et al.*⁸ have established the reality of this effect. Analysis of ice from near the firn line of the Storbreven in the Jotunheimen of Norway has revealed volume percentages of carbon dioxide up to ten times those for free air. Corroboration is provided by analyses of the composition of gas bubbles in Greenland icebergs by Scholander *et al.*⁹. These analyses showed that whereas the percentage oxygen content of the bubbles was in general lower than in free air, the carbon dioxide content was mostly higher.

As these results indicate, the properties of englacial gas bubbles change with time. Coachman *et al.*¹⁰ have made a systematic analysis of ice from the Storbreven between the firn region and the terminus. They found that although the gas pressures increased distally (downstream), the volume percentages of carbon dioxide decreased in the distal direction from three times those of free air to values very close to those of free air. This decrease is attributed to removal of the highly soluble carbon dioxide by means of meltwater leaking between the ice crystals by capillarity; a process which is fully compatible with the claim of Kers³ that it is "fossil" carbon dioxide which is responsible for the carbon dioxide enrichment of the pressure meltwater agent which exsolves and redeposits limestone subglacially.

The location and morphology of the subglacial limestones described by Ford *et al.* from the Rocky Mountains are consistent with a pressure meltwater hypothesis. The deposits are restricted to limestone benches. Development of pressure meltwater above a stoss face is to be expected and has been observed by Carol¹¹ beneath the Upper Grindelwald Glacier. Being under pressure and virgin, pressure meltwater enriched with fossil carbon dioxide would corrode with the maximum possible efficiency into the stoss face by which it was generated. The stoss faces which are now exsolved and "scoured into a drumlinized microtopography" may therefore have acted as sources of primary carbonate for subsequent lee side deposition. Ford *et al.* describe the occurrence on steep lee sides of short horizontal stalactites which adhere to tiny solutional groovings. These stalactites seem perfectly analogous to the tiny regelation spicules—also the products of pressure meltwater—observed beneath the Blue Glacier, Washington, by Kamb and LaChapelle¹². Moreover, both the laminated and the concretionary types of limestone deposit as described by Ford *et al.* have formerly been explained in terms of a pressure meltwater hypothesis³.

Thus the problems of genesis met by Ford *et al.* in attempting to explain the formation of the subglacial limestone deposits of the Mt Castleguard area of the Rockies may be overcome by supposing that the limestones are the products not of free meltwater but of pressure meltwater.

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stone outcrops, which may not have been the same areas that are exposed today, would have become peat covered.

During the early development of blanket peat the localized patches of oak and elm were largely unaffected and therefore an overall climatic determinant on the degeneration of the vegetation does not seem likely. Features in the pollen curves⁶, often thought to be indicative of climatic aberration¹⁶, could well be anthropogenic features, and they reflect continuing soil degradation, first started by man, under conditions of high precipitation.

The charcoal fragments point to fire being used by early man, perhaps the hunting and food gathering Mesolithic peoples of Boreal and Atlantic times, who accidentally or purposely cleared considerable areas during the hunting or coralling of game and made use of specific locations, such as springheads, as resting places during hunting expeditions^{7,17}. The resultant soil acidification would allow acidophilous vegetation to blanket peat development. Although the total activities of early man can only be surmised, his continued presence, from Atlantic times onwards, is indicated by the persistence of charcoal fragments. These peoples might well have exerted a strong influence on the vegetational development in an upland area where the soil was easily changed by rapid pedogenic processes, especially podsolization, after clearance of the existing vegetation¹⁸.

A principal feature of the Flandrian history of Cronkley Fell is continued instability since the late Glacial, both in terms of local mire dynamics and woodland history. This instability may be divided into phases: (1) the continuance of Late Glacial solifluxion^{7,19,20}; (2) ground water fluctuations in Zone VI^{8,3,21}; (3) the activities of successive prehistoric peoples who, by their burning, ensured the maximum tension between vegetation and soils¹⁸.

Table 1 Remains of Plants Found during the Time of the Maximum Forest Development on Cronkley Fell.

A. Rarer flowering plants and bryophytes²

Polygonum bistorta type
Saxifraga stellaris/*S. nivalis*
Helianthemum (*H. canum*)
Epilobium (*E. anagallidifolium*)
Rubus chamaemorus
Rubiaceae (*Galium boreale*, *G. pumilum*)
Camptothecium nitens

B. Ruderals or weeds of the Late Glacial⁸

Artemisia sp.
Centaurea cyanus
Chenopodium (*C. album*, *C. rubrum*)
Plantago lanceolata
Plantago major/*P. media* type
Potentilla (*P. anserinum*)
Ranunculus (*R. acris*, *R. repens*)
Urtica (*U. dioica*)
Rumex acetosa

C. Full Glacial or Late Glacial plants of open mountain or sub-arctic conditions⁸

Thalictrum (*T. alpinum*)
Selaginella selaginoides

Where the family or genus only has been found the rare species is enclosed in parentheses.

The period of maximum forest extension was obviously critical for the rarities, many of which are heliophytes. The presence of their remains, although scanty, along with the remains of Late Glacial ruderals (Table 1), indicates that sufficient habitats, primarily produced by environmental instability, allowed the persistence of communities characteristic of more open conditions. The postulate that the plants may have survived above the tree line, as Donner suggested for Ben Lawers⁴, must be questioned particularly as Pennington pointed out²² that on well drained mountain slopes trees could have existed up to 760 m.

Man may therefore have played an important part in the survival of the rare species during the time of blanket peat formation by creating unstable conditions and he may have been instrumental in the production of similar specialized communities to those which are found today on the margins of cultivated land³ and on mining spoil heaps²³.

There would seem sufficient data to suggest: (1) environmental conditions have never been totally inimical to the persistence of the Teesdale rarities throughout the Flandrian period; (2) the rarities are indeed relics from Late Glacial times although man has played a considerable part in the promotion and extension of habitats in which the plants were able to survive.

This is a summary of the research undertaken during post-graduate study. A fuller report will be presented in due course. This work was financed by the Natural Environment Research Council. I thank Dr I. G. Simmons for supervising the work and Dr D. J. Bellamy for identifying the moss remains.

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Interpretation of the 'Massenerhebung' Effect on Tropical Mountains

THREE types of rain forest can generally be recognized on wet tropical mountains: lowland rain forest, lower Montane rain forest and upper Montane rain forest¹⁻³. These forest types can be defined both by distinctive plant associations¹ and by the altitudinal limits within which they lie. These limits, however, vary with the type of mountain. On small, isolated mountains and outlying ridges of major ranges, the upper limit of lowland rain forest is about 700–900 m and that of the lower Montane rain forest about 1,200–1,600 m, whereas on the main ridges of major ranges the limits are higher, approximately 1,200–1,500 m and 1,800–2,300 m, respectively⁴. This phenomenon is known as the 'Massenerhebung' effect.

The forest types are related to the amount of fog cover; lower Montane rain forest is correlated with frequent fog, and upper Montane rain forest with very frequent and more persistent fog⁴. No explanation has yet been given for why the forests should be correlated with frequency of fog. Response to temperature does not seem to be a contributory factor, for there is no evidence of a greater lapse rate on smaller mountains^{4,5}. Incident light intensity is decreased in fog-bound forests, but for seedlings regenerating in the undergrowth this effect may be largely offset by the lower and generally more open canopy of the Montane forest types¹; thus it seems unlikely that light intensity controls the distribution of the major forest types. Poor aeration of the soil may be important in limiting upward extension of lowland or lower Montane species into the upper Montane environment⁶ but it is probably not sufficiently marked to be effective at the lower boundary of the lower Montane forest; marked peatiness of the soil and gleying are only found toward the upper limit of lower Montane forest^{7,8}.

An explanation of the Massenerhebung effect is given here in terms of the availability of certain nutrients. The content of organic matter in the soil increases markedly with altitude⁷⁻¹⁴. This increase can follow either from decrease in temperature or from increase in soil water content^{9,11}. This suggests that the rate of mineralization of the humus decreases with either lower mean temperature or increase in soil water content¹⁰. It is generally held that there is a nearly closed cycle of nitrogen and mineral nutrients in tropical lowland rain forests^{1,15,16}. If this is true of tropical Montane rain forests, the nitrogen and phosphorus available to the plants must be provided largely by mineralization of organic matter and it is reasonable to suppose that these elements will be in lesser supply if either mean temperature decreases or frequency of fog increases. It may be that mineralization is slower in the Montane forests partly because the litter is inherently less easily decayed; litter from trees of less fertile sites in northern temperate forests is generally less easily decayed^{17,18}. It may also be that substances which inhibit the bacterial breakdown of humus are leached from the canopy or litter of Montane forests; this seems to be the case with forests on certain infertile soils of the north temperate zone¹⁹. The hypothesis set out below is equally valid whether the slower mineralization is wholly induced by the physical factors of the environment or partly induced by the properties of the plants.

It is suggested that the uppermost limits ever reached on tropical mountains by lowland rain forest plants are governed by temperature effects, as are the latitudinal limits in the lowlands in the one country where drought is not the dominant limiting factor, that is, in southern China²⁰. The transition there from tropical lowland rain forest to warm temperate rain forest (oak-laurel forest) coincides with a mean temperature of about 20°–22° C. It is not known whether the critical function of temperature concerns the amount of warmth in the warmer months or amount of cold in the colder months; perhaps both are involved. The effects of temperature are presumably in part direct (on rates of photosynthesis, respiration and morphogenesis) and in part indirect (on rates of mineralization).

It is suggested that the upper limit of lowland rain forest plants can be brought down to varying degrees below the temperature-determined uppermost limit, when the fog characteristic of the lower Montane environment occurs at lower levels, because this raises the soil water content and intensifies the slowing down of the mineralization of organic matter. This is the cause of the Massenerhebung effect. The poorer supply of nitrogen and phosphorus is likely to have a strong effect where temperature is already beginning to be unfavourable. In northern Europe many cases are known in which there is an intensification of the effects of poor mineral supply where temperature and light supply become increasingly unfavourable²¹. Possibly the temperature-determined uppermost limit for at least some lowland rain forest species on

tropical mountains is almost never reached, even in the most massive ranges, because of the effects on mineralization of the fog characteristic of the lower Montane environment.

It is suggested that the uppermost limits of lower Montane and upper Montane rain forest plants are set similarly by temperature and that these limits can be brought down to varying degrees by fog as this reduces both the supply of nitrogen and phosphorus and the degree of soil aeration. Low availability of copper or zinc, because of binding in the highly organic soil²², may also be important.

The lower limits of upper Montane and lower Montane forest plants are likely to be governed as much by competition from plants of the next forest type down as much as by direct effects of the physical environment because in general the environment becomes more favourable for plant life with decrease in altitude. Some support for this view is provided by the occurrence of various upper Montane species in the lowlands on sites with an exceptionally poor mineral supply and a low, open canopy³. The higher temperatures in the lowlands may well be lethal for many species, however, as they are said to be for such crops as quinine, coffee and tea²³; susceptibility to drought stress may also be important²⁴.

The increasingly poor supply of nitrogen and phosphorus dependent on decrease in temperature and increase in frequency of fog is likely to be important not only in governing the distribution of forest types but also in influencing their biomass and physiognomy. The mean leaf size decreases and the frequency of sclerophylly increases with altitude¹; these features are known to be related to shortage of nitrogen and phosphorus in other situations²⁵⁻²⁹. The decrease in biomass¹ may also reflect in large part the slower turn-over in these principal nutrients.

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Role of Temperature in the Regulation of Leaf Extension in *Zea mays*

ALTHOUGH innumerable experiments have indicated the extent to which plant growth is dependent on temperature, much remains to be discovered about the mechanisms underlying these responses, and about the temperature sensitivity of different plant organs.

Brouwer¹ suggested that leaf expansion could be influenced by soil temperature through the water supply to the leaf tissue. More recently, experiments on *Zea mays* by Beauchamp and Lathwell² and by Kleinendorst and Brouwer³ have indicated that the temperature of the shoot apical meristem may be more important than that of the root system. In the present work, leaf extension was recorded continuously while sudden changes were made in the temperatures of the root system and of the shoot meristematic region. In experiments of 2–3 h duration, two separate responses were found.

Rates of leaf extension were measured using an auxanometer similar to that described by Williams and Biddiscombe⁴, making it possible to detect changes in rates of extension within two minutes. Plants of *Zea mays* L. (cv. 'Erliking' F₁ hybrid) at the third leaf stage were set in rubber bungs so that the apical meristem of the shoot projected just above the surface of the rubber. Collars made from 'Perspex' tubing 25 mm in diameter and 20 mm in height were sealed to the bungs with silicone rubber compound so as to encircle the shoot meristem. These collars were filled with water. The temperature of the meristematic region could thus be varied independently of the root or shoot system by passing a small current through resistance wire wound round the collar or by circulating chilled water inside the collar.

The plants were suspended with their roots in nutrient solution in a temperature controlled water bath, whilst the shoots were illuminated in air at 25° C and either 50 or 100% relative humidity. The light intensity was 40 W m⁻² from warm white fluorescent lights.

Preliminary experiments revealed that the elongation zone of the youngest (third) leaf was confined to the 20 mm section contained by the meristem collar. When the temperature of either the root system or the shoot meristematic zone was altered, the rate of elongation of the third leaf began to change within 2–3 min, and reached a new constant value within

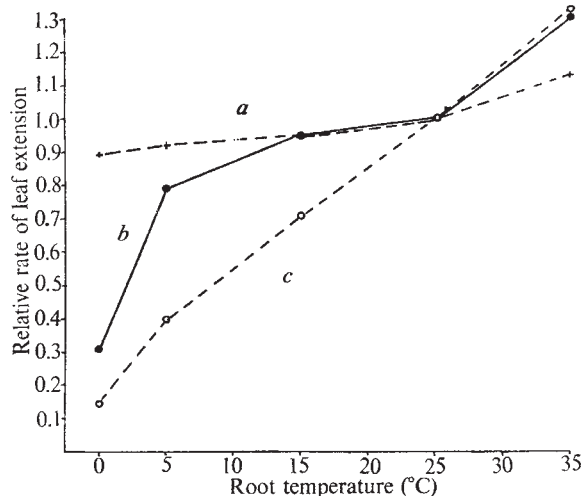


Fig. 1 Relative rates of leaf extension at root temperatures 0 to 40° C, when the temperature of the meristematic region is maintained at 25° C, and when it is allowed to vary with root temperature. ○ --- ○, Meristem temperature allowed to vary with changes in root temperature, relative humidity 50%; ● — ●, meristem temperature kept at 25° C, relative humidity 50%; + --- +, meristem temperature kept at 25° C, relative humidity 100%.

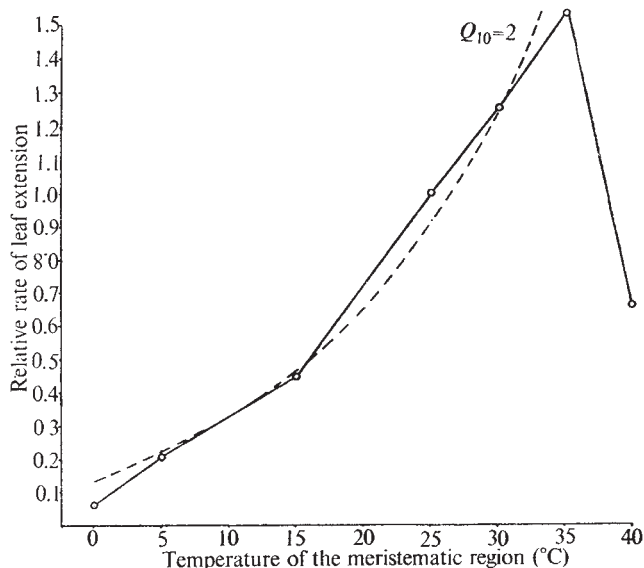


Fig. 2 Relative rates of leaf extension when the temperature of the meristematic region is changed from 0° to 40° C, and the temperature of the root zone and the air around the leaves is kept at 25° C. A curve with a Q_{10} of 2 (---) is fitted to the experimental results. ○ — ○, Meristem temperature changed from 0° to 40° C, root zone and air temperature kept at 25° C, relative humidity 50%.

15 min. The response to changes in root temperature was too rapid to have been a result of changes in the pattern of nutrient uptake, or growth hormone distribution, but was consistent with the restriction of water uptake at low temperatures observed by Kuiper⁵ and others. Measurements with a thermocouple psychrometer, however, showed that leaf water potential and pressure potential were independent of root temperature between 5° and 25° C, and did not decrease until the temperature was lowered below 5° C.

When the temperature of the meristematic region was held at 25° C in the absence of transpiration (100% relative humidity), lowering the root temperature from 25° to 0° C caused a decrease of only 10% in relative leaf expansion (Fig. 1, curve *a*). If transpiration were permitted (50% relative humidity), however, the curve was of a plateau type (Fig. 1*b*), the rate of leaf expansion being very low at 5° C, but much faster at 35° C than at 25° C. Clearly the thermostatic collar could not completely overcome the heating or cooling effect of the transpiration stream from the roots on the shoot meristem and the zone of expanding cells. The sharp decrease in expansion rate from 5° to 0° C was ascribed to the more negative water potentials of leaf tissue observed between these temperatures. When the temperature of the apical meristem and the zone of expanding cells was allowed to follow passively that of the root system, Fig. 1*c*, the rate of elongation decreased by an order of magnitude between 35° and 0° C, presumably because the conduction of heat to and from the meristematic region by the transpiration stream was now reinforced by conduction through the tissue of the mesocotyl. At temperatures below 5° C there was a sharp decline attributable to water stress.

Fig. 2 shows the rates of elongation of the third leaf when the temperature of the meristematic region was varied and the root and shoot temperatures held constant at 25° C. Over the temperature range 5°–30° C, the rate of leaf extension doubled for each 10° rise in temperature. Comparison of curve *c*, Fig. 1, with the response to meristem temperature in Fig. 2 suggests that when the root temperature was 15° C, the meristem temperature was about 20° C.

Changes of temperature between 5° and 25° C in the shoot meristematic region do not affect water uptake³. The Q_{10} value of 2 in Fig. 2 suggests that the manufacture of new tissue

may be limited by the rate at which energy becomes available from the products of respiration, at least between 5° and 30° C.

In the present experiments, the temperatures of the root and of the meristematic region were found to act and interact in the regulation of leaf expansion. Other experiments, to be reported elsewhere, will show that changes in shoot temperature also produce specific responses independently of root and meristem temperatures. The expansion of young maize leaves is therefore sensitive to (a) the temperature of the leaf tissue, (b) the rate of transpiration, (c) the meristem ambient temperature, (d) the temperature of the root system in transpiring plants, and (e) the water status of the tissue in the meristematic regions when the root zone is cooled below 5° C.

If the behaviour of other species is equally complex, conventional phytotron experiments relating plant growth to temperature will yield little information relevant to field conditions, for most phytotrons are designed to keep the whole plant at the same temperature. To understand how plants respond to natural microclimates, growth must clearly be studied in relation to differences in temperature between different plant organs.

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Long Term Persistence of Parathion in Soil

PARATHION, an early organophosphorus insecticide introduced commercially in 1947, is, like most other compounds in this group, considered to be relatively non-persistent on plants and in soil. West¹ reported its persistence to be 5–12 days on plants, and Lichtenstein² showed that 97% of a soil application disappeared within three months.

Field plots, established at Kentville, Nova Scotia, in 1949 to study the persistence of parathion in soil and its uptake by plants, received annual spring applications of 31.4 lb per acre (15.7 p.p.m. soil concentration) of parathion from 1949 to 1953 inclusive. The compound was thoroughly incorporated to a depth of 6 inches with a rotary cultivator and various crops have been grown each year up to the present.

Parathion residues were at first determined by the relatively non-specific colorimetric method of Averell–Norris³. Chisholm *et al.*⁴ found that parathion disappeared rapidly from the soil, the concentration after five annual spring applications of 15.7 p.p.m. being only 0.5 p.p.m. in the autumn of 1953. By 1964 the soil concentration had fallen to 0.2 p.p.m. The Averell–Norris method, however, does not distinguish between parathion and degradation products containing aromatic nitro or amino groups.

The acquisition of a flame photometric detector⁵ prompted a reinvestigation of these plots in an attempt to identify the compound or compounds in the soil responsible for the characteristic magenta colour produced by the Averell–

Norris reagent. Soil samples representative of the 0–4, 4–8, 8–12 and 12–16 inch depths were collected from each of four plots in the spring of 1969. After air-drying at room temperature the soils were extracted with hexane–acetone (1:1) in a Soxhlet extraction apparatus, and the extracts concentrated to 2 cm³/g of soil. Preliminary observations, using a Tracor MT-220 gas chromatograph with a Melpar flame photometric detector and 3% OV-17 column, indicated that a substance containing phosphorus and sulphur, with a retention time identical with that of parathion, was present in the soil at a concentration of about 0.1 p.p.m. Further work using a 2% DEGS column and a 10% DC-200 column confirmed that this substance was indistinguishable from parathion by gas–liquid chromatography. No other sulphur or phosphorus-containing compound was detected in the extracts.

The relative phosphorus–sulphur response given by this substance in the flame photometric detector was the same as that of parathion. Its distribution coefficients⁶ in three solvent systems (hexane–acetonitrile; heptane–90% ethanol; iso-octane–80% acetone) were also identical with those of parathion. Finally, the presence of parathion in this soil was confirmed by thin layer chromatography of soil extracts on silica gel plates using several solvent systems. Parathion was detected on the plates by an esterase inhibition technique⁷ and the flame photometric response of material eluted from the appropriate position on the chromatogram. Several solvent systems acetone; hexane–acetone (1:1); methanol–chloroform (1:1) extracted almost identical amounts of parathion from the soil. Methanol extracted smaller amounts. Addition of one part water to nine parts air-dry soil before Soxhlet extraction did not improve extraction yields.

During the period 1949–69 there was little downward movement of parathion in this sandy loam soil (sand 57%, silt 15%, clay 28%, organic matter 3.7%, pH 6.2) even though the average precipitation was 42 inches per year (Table 1). Samples taken outside the perimeters of the plots indicated a small amount of lateral movement which appeared to be a result of cultivation.

Table 1 Parathion Residues in Experimental Plots (1969)

Depth (inch)	p.p.m. Parathion*
0–4	0.060
4–8	0.063
8–12	0.008
12–16	Trace

* Average of four replicates.

About 0.1% of the total parathion applied to the plots remained in 1969, 16 years after the last application. The reason for this long persistence in soil, hitherto unreported for an organophosphorus pesticide, is unknown. It is possible that parathion may be dissolved in lipids of the soil organic matter and thus be protected from bacterial degradation and hydrolysis.

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Specific Chromosome Anomaly associated with Autonomous and Cancerous Development in Man

SEVERAL studies¹⁻⁴ have now shown that chromosome variation in cancer cells is non-random, and that during cancer progression at least natural selection favours certain chromosome types while others are eliminated. Similar patterns of non-random representations of chromosome types have also been reported in established cell lines⁵⁻⁸.

Significant gains have been reported in the *C* group chromosomes (Denver classification) and, not surprisingly, in "marker" chromosomes, and significant losses are found in the *D* and *G* groups. This selection is not entirely a reflexion on the stability of chromosome groups during prolonged growth *per se*, because in leukaemia the best studied cases of chronic myeloid leukaemia (CML), acute myeloid leukaemia (AML) and acute lymphatic leukaemia (ALL) have all shown gains in *G*-chromosomes in hyperdiploid cells⁹.

In all these studies the Denver classification has been used. This method of classification is adequate where only one or two aberrations can be expected, such as in constitutional karyotypes where the cells have been through embryogenesis. When applied to cancer cells, however, the analysis means squeezing the chromosomes into the Procustes bed of Denver in a most crude manner. Furthermore, the Denver system with regard to length distinction between the groups is not nearly so clear in the myeloid and erythroid series and other tissue cells, as it is in lymphocytes on which the Denver system is based.

To examine in detail the over and under-representation of chromosome types, a small selection of neoplasms of exceptional technical quality has been studied by a computer method which makes possible an unbiased assessment of the proportional representation of chromosome types in cancer cells. The method eliminates all decision-making, by automatic grouping of the chromosomes on centromere indices—six types (*M*: 0.50–0.45; *m*: 0.45–0.39; *mt*: 0.39–0.28; *st*: 0.28–0.16; *t*: 0.16–0.05; *T*: 0.05–0)—and on length—three sizes (*l*: greater than 5.97; *m*: 5.97–2.44; *s*: less than 2.44). The chromosomes are measured from enlarged prints by an *xy*-coordinate reader and sorted automatically into 18 "boxes" per cell. They are named by the centric index and size; that is, a chromosome with centromere position *mt* and of medium size is referred to as an *mtm* chromosome. The chromosomes are pre-labelled in the prints with consecutive numbers and measured in this order. These numbers are retained during data processing so that it is possible to refer back to the photographs for detailed examination.

Normalization was carried out by adding or subtracting the respective number of average chromosome lengths to the normalization number for the diploid cell (male 207.70; female 210.94). Control measurements were obtained from normal lymphocytes and from selected samples of normal cells with different degrees of chromosome contraction. The three empty boxes in normal cells (*Tl*, *Tm*, *Ts*) were used for storing acentric fragments and other aberrations which were included in normalization since they affect the genetic balance of the particular cell.

Five effusions were studied, either serially or in different regions of the range of chromosome counts. The data will be fully described elsewhere. The simplest way of presenting the data, consisting of 16 subsamples, is to present the percentage chromosome type in each box arranged according to descending order in the control. All measurements were repeated in the 16 samples with a total of 60,000 chromosome measurements. The most significant findings are presented in the graph (Fig. 1). A significant gain was found in all samples in the *Mm* box with an average of 11.73% whereas the control cells showed only 5.54%. Losses of chromosomes are found in the *ml* and *tm* boxes and in almost all *ts* and

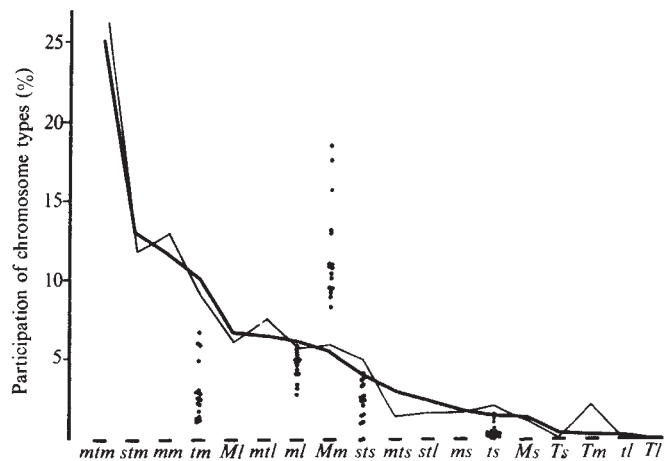


Fig. 1 Graphic representation of the most significant deviations from the contour line of controls (—). Selected sample (see text) is shown as the thinner line. Sixteen samples of malignant cells are shown by their means presented as dots. All samples show *Mm* gains and the losses are confined to boxes contributed to by the acrocentric *D* and *G* group chromosomes, except for a smaller but consistent loss of *A* chromosomes from *ml*.

sts boxes. Although present in all samples, the *ml* (*A* group in controls) loss is slight, 4.61% against 6.74%. The *tm* losses (*D* group) were found in all samples and the difference was significant, 3.07% against 10.11%. *ts* loss occurred in 15 samples, and the average value for the box was 0.53% against 1.47%. This box contains *G* chromosomes which overspill into *sts*, also showing loss, 2.33% against 3.97%. Apart from the losses of *A1* and *A3* chromosomes, therefore, the *D* and *G* chromosomes were consistently involved in losses. Both these losses and gains were progressive in serial samples.

It is clear that in previous studies^{3,4} the *Mm* box chromosomes have been referred to the *C* group. On inspection of the photographs of the *Mm* a number of translocations of *G/G* type were found. These were particularly clear when dicentric with a short inter-centric distance not exceeding twice the short arms of *G* chromosomes. Some intercentric distances were short, however, and could only be observed as the extra length of the centromere constriction. Because of the reduced length differences between groups in cancer cells compared with normal lymphocytes, it is not surprising that *G/G* translocations may be included in the *C* group. The short intercentric distance makes it unlikely that they often have been singled out as "markers". These dicentrics are capable of propagation. From inspection of the photographs, many *D* and *G* chromosomes seem not to have clear short arms, suggesting either that they have undergone deletions or that they are not in fact *D* and *G* chromosomes.

We have only looked at "net" gains and losses, so that if chromosomes are lost from a group which is also gaining chromosomes, the fluctuation may not be revealed. This may be the case with box *ml* (and *Ml*), containing *A* chromosomes, to which *D/D* translocations contribute. *D/G* translocations give chromosomes indistinguishable from *C* chromosomes except when dicentric.

Chromosome 16 falls into the *Mm* box. Among the spontaneous abortions^{10,11}, chromosome 16 represents perhaps the most common trisomy. It is therefore conceivable that extra 16s may be present in box *Mm*, and they may also be selected for. But the excess in the box in the case of malignant cells comes mainly from iso-chromosomes and translocations, particularly those involving the acrocentrics (centric fusion). These are also the most common "spontaneous" translocations in man, although limited to one per affected karyotype. These can be dicentric. The translocation forming lesions occur on both sides of the centromere, so there could be at least

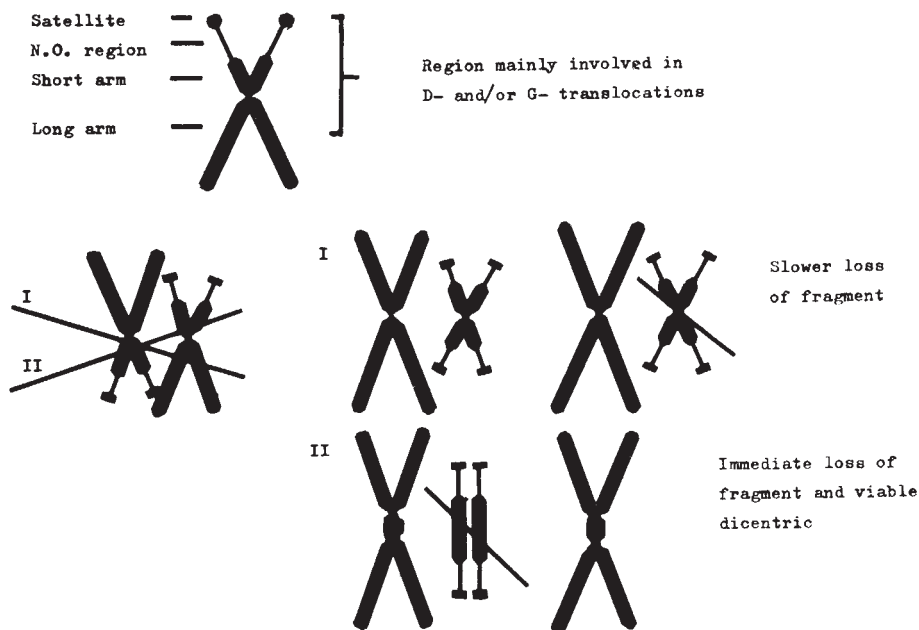


Fig. 2 The most common translocation types involving *D* and *G* chromosomes. I, Exchange positions resulting in formation of two centric translocation chromosomes; II, exchange positions resulting in a dicentric chromosome and an acentric fragment. Dicentrics with long intercentric distance which tend to start 'breakage-fusion' cycles are not formed from these translocations.

twice as many translocation chromosomes as the observed incidence of dicentrics of this type.

Such translocations, however, may lead to a specific effect—namely the systematic and progressive loss of normal nucleolar organizers—NOs—which seems to be a consistent finding in malignant cells and "established" cell lines. For each dicentric translocation there is a corresponding acentric fragment carrying both NOs (Fig. 2). This fragment is immediately lost, as are fragments produced by iso-chromosome formation from acrocentrics. The mono-centric translocation chromosome should produce a complementary mono-centric fragment with both NOs. Inspection of the fragments shows that all satellited fragments must be rapidly lost. Only some of the possible *D/D* and *D/G* translocations have been demonstrated in constitutional carriers¹².

Assuming that each NO region is specific for the chromosome type, we have 5 different NOs in the human complement. Translocations between homologues will lead to the irrevocable loss of a specific NO, while translocations between non-homologues will still leave the respective NOs of the intact chromosomes which might compensate by increased activity. If all NOs are identical their differences in lengths might arise from variations in repeats of rDNA cistrons, and the effect of their loss might be quantitative.

We propose that tumour cells contain *D* and *G* translocations which are not known constitutionally, and that they have consequently lost the corresponding NOs present in normal cells. If this loss is relevant to cancer, translocation carriers (of this type) would have an increased tendency to develop tumours or develop more malignant ones.

It is not yet possible to differentiate between the products of these five NO regions. With a suitable technique, however, translocation carriers with autoradiographically identified translocation chromosomes—and therefore with known NOs missing—might be tested for difference in their rRNA.

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Uterine Changes during Capacitation in the Golden Hamster

'CAPACITATION' was defined by Chang¹ as the final maturation of spermatozoa in the female genital tract. Since Chang's report considerable research has been directed towards this phenomenon^{2,3}. Capacitation has been observed outside the female tract in such areas as the eye or urinary bladder⁴ as well as *in vitro* with follicular fluid⁵ or eosinophils⁶. The viability of ejaculated sperm is determined by factors such as levels of oestrogen and progesterone⁷, and the phase of the oestrous cycle, including the different positions in the female genital tract⁸. Hunter⁹ has recently demonstrated in hamsters that ejaculated spermatozoa exposed to oestrous uterus underwent capacitation with a high rate of zona penetration. He concluded that an incubation effect occurred directly between the uterine environment and the spermatozoa. Even though the epididymal sperm were capacitated, they were not very fertile⁹. It seems that for normal fertilization the uterine environment and seminal plasma are indispensable factors. In studying mammalian fertilization I became interested in the role of the uterine epithelium in capacitation. This preliminary report describes uterine changes during capacitation. A detailed report will be published elsewhere.

Thirty adult female hamsters *Cricetus auratus* were mated and their reproductive tracts were fixed at 30 min intervals starting from zero to 300 min after mating. Sections were stained with Mallory's triple stain and PAS-haematoxylin. Acetone fixed materials were used for the detection of alkaline phosphatase concentration using Gomori's method¹⁰. Epithelial cell height was measured by ocular micrometer.

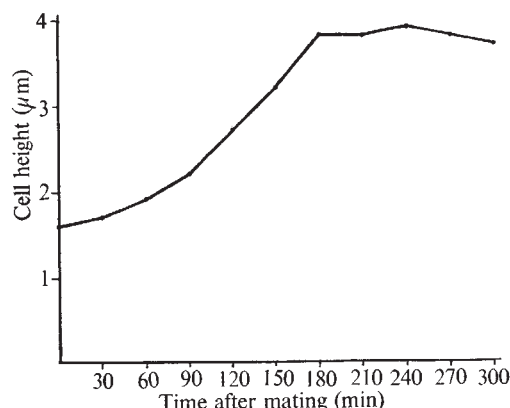
The PAS positive secretion of the uterine epithelium was seen to increase gradually after mating. The secretion was

Table 1 Reaction of the Uterine Epithelium for Alkaline Phosphatase and PAS Positive Mucopolysaccharide in Hamsters following Mating

	Time (min) after mating						
	30	60	90	120	180	240	300
PAS positive mucopolysaccharide	Faint	Moderate	Moderate	High	Intense	Intense	Intense
Alkaline phosphatase	Faint	Moderate	Intense	Faint	Very faint	Very faint	Very faint

resistant to 'diastase' and seems to be a mucopolysaccharide-protein secretion¹¹. A high concentration of PAS positive material was noted within 150 min of mating. There was no apparent change in PAS reaction in the uterine epithelium between 150 min and 300 min after mating. Conversely, alkaline phosphatase activity, as judged by the blackness of the tissues, was different. Tissues fixed 30 min and 60 min after mating showed good (positive) reaction with 15 min of incubation. Tissues fixed 90 min or later after mating exhibited faint or poor reactions; prolongation of incubation up to 45 min did not give a good reaction (Table 1).

Uterine epithelium undergoes a morphological change after mating. Thirty min after ejaculation the height of the columnar epithelium began to increase and reached 3.8 μ m 180 min after mating (Fig. 1). Thereafter no marked increase in height of the cells was observed.

**Fig. 1** Increase in height of the uterine epithelium after mating.

Two important points emerge from this investigation. First, mucopolysaccharide secretion and height of cells gradually increase in the uterine epithelium of hamsters after mating. Second, alkaline phosphatase concentration decreases 90 min after mating. Proteinogenous secretion and alkaline phosphatase of the uterus appear to contribute factors necessary for the capacitation and metabolism of sperm in the hamster. These observations lend support to the views that enzymes^{12,13} and "decoating" factors¹⁴ aid in the sperm capacitation of mammals.

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Rupture of the Aorta produced in the Hamster by Anti-ovulatory Progestogens

MEGESTROL acetate (17 α -acetoxy-6-methylpregna-4,6-diene-3,20-dione) (BDH 1298) and its C₁₆-methylene analogue melengestrol acetate (17 α -acetoxy-6-methyl-16-methylenepregna-4,6-diene-3,20-dione) (BDH 1921) are steroids with high progestational and anti-ovulatory activity and with little or no oestrogenic and androgenic properties. Megestrol acetate is used in women for oral contraception^{1,2} and both megestrol acetate and melengestrol acetate are being investigated for possible use in the treatment of certain hormone-dependent tumours^{3,4}. During a study of the activity of these drugs against an oestrogen-dependent renal adenocarcinoma⁵ in the hamster, animals frequently died of rupture of the aorta. Some ruptures occurred spontaneously and others during routine handling of the animals. This phenomenon was investigated further in non-tumour bearing animals.

Table 1 Number and Sex of Animals dying from Ruptured Aorta in Relation to Treatment with Melengestrol Acetate and Megestrol Acetate

Dose given subcutaneously twice weekly for 8 weeks	Treated				Control			
	Melen- gestrol acetate		Megestrol acetate		Solvent		Un- treated *	
	M	F	M	F	M	F	M	F
4 mg/kg	0/5	0/4	0/4	0/4				
20 mg/kg	3/5	0/4	0/5	0/5				
100 mg/kg	2/4	2/4	1/8	0/8				
200 mg/kg	3/5	1/5	0/4	1/4				
Totals	8/19	3/17	1/21	1/21	0/5	0/5	0/8	0/8

* The untreated control hamsters received no injections at all and were weighed weekly at the same time as the other animals in the experiment.

Male and female golden hamsters were injected subcutaneously twice weekly for 8 weeks with either megestrol acetate or melengestrol acetate at various dose levels (Table 1). The steroids were suspended in a solution of carboxymethylcellulose (0.5%), polysorbate (0.5%) and benzyl alcohol (1%). The experiment was terminated after 8 weeks of treatment. Groups of solvent-treated, and normal untreated, control animals were also studied (Table 1). The heart and aorta were dissected out of the animals as soon as was possible after spontaneous death or when they were killed at the end of the experiment. The tissues were fixed in Bouin's solution and stained with haematoxylin and eosin, Verhoeff (for elastic fibres), congo red (for amyloid) and Gram's stain (for micro-organisms).

Fifteen of the 36 animals treated with melengestrol acetate died before the end of the experiment at 8 weeks. Eleven of the fifteen died from rupture of the aorta, the remaining four from inanition of unknown origin. Two of the 42 animals treated with megestrol acetate died before 8 weeks, both from rupture of the aorta. With both drugs the deaths occurred between 15 days and 8 weeks. At autopsy a large blood clot

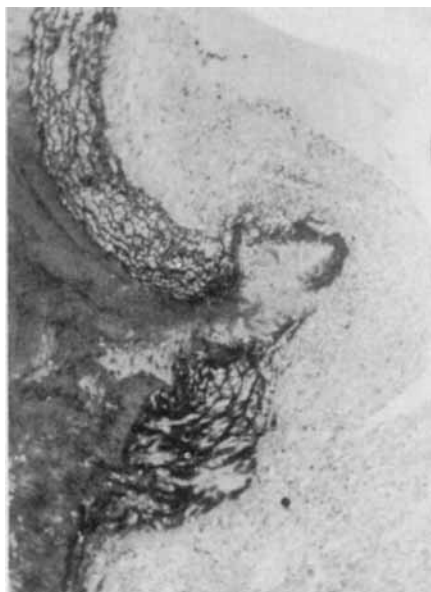


Fig. 1 Rupture of the wall of the aorta just above its origin from the left ventricle. Stained by Verhoeff's method for elastic fibres ($\times 60$).

was seen at the base of the heart, except in one case treated with melengestrol acetate where the heart was normal and fatal haemorrhage had taken place around the aorta at the level of the renal arteries. The lesion at the base of the heart was located in the wall of the aorta close to its origin from the left ventricle (Fig. 1). The site of the rupture was invariably characterized by local necrosis of the tunica adventitia and tunica media. The reaction was usually nodular and the nodules, which measured up to 200 μ m in diameter, frequently had necrotic centres. The area involved in the reaction could extend along the atrio-ventricular septum to the origin of the pulmonary artery. A constant feature of the rupture was a complete severance of the elastic fibres of the media (Fig. 1). The condition of the aorta before rupture could be gauged from the histological appearance of areas of the vessel away from the actual rupture showing nodular necrosis of the adventitia. Degeneration of the elastic of the underlying tunica media in these areas gave it a lace-like appearance.

Nodular necrotic lesions were also seen in six of the sixteen untreated control animals, and in two of the ten solvent-treated controls. The lesions occurred at the origin of the aorta from the left ventricle and around the renal arteries and adjacent aorta. They looked similar to those seen in treated animals with the exceptions that the underlying elastic fibres appeared intact and there was no rupture of vessel walls.

The cause of the nodular inflammatory reaction in the adventitia of the aorta and renal arteries in the control and treated hamsters is not known. Gram's stain did not reveal micro-organisms in the lesions. To a certain extent the arterial lesions resemble polyarteritis nodosa in man.

In five of the twelve animals with ruptured aorta, death occurred during routine handling for weighing and injecting. No animals in the control groups died from aortic rupture although they were handled in an identical way.

Rupture of the aorta in the treated animals may have been produced by exacerbation of an existing pathological change identical to that seen in the tunica adventitia of the aorta of control animals. The exacerbation may have arisen because of the anti-inflammatory activity of these steroids. The cotton pellet granuloma test in rats⁶ showed both drugs to have some anti-inflammatory activity. On a weight for weight basis melengestrol acetate is more effective than megestrol acetate in reducing inflammation. Rupture of the aorta has been observed in hamsters treated with cortisone⁷ and deoxycorticosterone combined with F cortisol⁸.

In these experiments megestrol acetate was injected subcutaneously at a dose rate approximately 100-fold greater than that taken orally for contraception in women. On the other hand, the doses of melengestrol acetate and megestrol acetate used for treatment of carcinoma in man is of the same order as that used in the present experiments.

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Host Specificity of Brazilian Strains of *Pasteurella pestis*

WE have examined eleven strains of *Pasteurella pestis* isolated by M. Bahmanyar, Y. Karimi and C. Rodrigues de Almeida in an inveterate focus of plague at Exu, Pernambuco, Brazil, and sent to us by M. Baltazard. We have confirmed the curious behaviour observed by these investigators, with all of nearly 200 such isolates from this focus, that Exu strains show high pathogenicity for mice but apparently are innocuous for guinea-pigs (M. Baltazard, personal communication). We have tried to explain this host-specificity by comparing the behaviour of these isolates with that of a typical fully virulent strain MP6, which is highly pathogenic for both these species.

From many tests *in vitro* the Exu isolates were found to differ from strain MP6 only in their lower growth rate on defined minimal agar¹. They showed normal growth rate, however, when this medium was supplemented specifically with L-asparagine. Recognition of their semi-dependence on asparagine for growth suggested an explanation for the low pathogenicity of Exu organisms for the guinea-pig, for this species, like other members of the *Cavioidae*, has a potent circulating asparaginase^{2,3} and consequently would present Exu organisms with an asparagine-free medium. Their subnormal growth rate *in vivo* could allow the guinea-pig to contain the infection for a period sufficient for the development of an active immunity. We routinely challenged guinea-pigs 21 days after Exu infections and invariably found them immune to doses of one million MP6 organisms intraperitoneally, indicating that Exu organisms multiply *in vivo* to give immunogenic populations.

The relative growth rates of Exu and MP6 organisms on defined minimal agar supplemented with mouse, human or guinea-pig bloods, with and without asparagine addition, are

Table 1 Relative Growth Rates of MP6 and Exu Organisms on Blood Media supplemented with Asparagine

Medium	Relative growth rate			
	MP6		Exu	
	Asparagine supplement	Asparagine supplement	Asparagine supplement	Asparagine supplement
	—	+	—	+
Minimal agar (MA)	1.00	0.95	0.74	1.00
MA+mouse blood	1.48	1.43	1.21	1.43
MA+human blood	1.48	1.48	1.21	1.54
MA+guinea-pig blood	1.53	1.48	0.83	0.83

Slopes of defined minimal agar, supplemented as shown with blood to give 20% and with L-asparagine to give an additional 40 µg/ml., were surface inoculated with 10^6 organisms and incubated at 28° C. Viable counts were made after 0, 18, 42 and 66 h. Growth rates were assessed from the slopes of growth curves over the exponential growth period 0–18 h and are quoted relative to that of MP6 organisms on MA. The growth rate of 1.0 corresponds to a mean generation time of 3.0 h. All treatments were duplicated.

given in Table 1. The data show that mouse and human bloods contain levels of asparagine which are sub-optimal for growth of Exu organisms at the higher rate shown by MP6, and that addition of asparagine to guinea-pig blood fails to accelerate the growth of Exu organisms. On agar supplemented with guinea-pig blood, heated to 80° C for 30 min (to inactivate contained asparaginase) and then supplemented with asparagine, organisms of both strains grew at the high rate shown by MP6 on unheated medium.

The concentration of asparagine in human blood was estimated by treating with asparaginase (20 IU purified asparaginase/ml. blood for 16 h at 37° C), heating to 80° C for 30 min, adding back known amounts of asparagine and noting that concentration which permitted Exu organisms to grow at the rate obtaining with untreated blood. These assays indicated the level of asparagine to be near to 10 µg/ml. (using a pool of nine samples) and that a level of 20 µg/ml. was required to permit Exu organisms to grow as rapidly as MP6. Using chromatography, Stein and Moore⁴ found the free asparagine in human blood plasma to be 6 µg/ml. The similar behaviours of mouse and human bloods in growth tests with Exu strains indicate that they have similar asparagine contents. It is probable that the increase in mean time to death of about 1 day observed in mice infected with Exu organisms, compared with those infected with similar low doses of MP6, results from the sub-optimal level of available asparagine.

Table 2 Delaying Effect of Asparaginase on Death from Exu Infections in Mice

Treatment of mice	Infecting organism			
	MP6	Exu	MP6	Exu
	Mortality (%) [*]	Mean time to death (days)	Mortality (%)	Mean time to death (days)
Nil	100	3.5	100	4.5
Asparaginase (2,000 units)	100	2.8	90	5.8
Asparaginase (5 × 400 units)	100	3.1	90	7.7
Guinea-pig serum (0.2 ml.)	100	3.7	80	4.7
Guinea-pig serum (5 × 0.2 ml.)	100	3.8	100	6.4

* Groups of ten mice per treatment; observed 21 days.

Mice were injected intraperitoneally with 10^3 MP6 or Exu organisms. After 24 h they were injected intraperitoneally with 0.2 ml. saline containing 2,000 units of 'Porton L-asparaginase' (specific activity 590 IU/mg) or with 0.2 ml. containing 400 units followed by an equal dose on each of 4 successive days. Mice received 0.2 ml. guinea-pig serum immediately after infection, or then followed by equal injections on 4 successive days.

Asparaginase injected into mice infected with Exu organisms did not materially reduce mortality but still further increased the mean time to death while having negligible effect on infections with MP6. Injected guinea-pig serum similarly delayed death from Exu infections (Table 2). The failure of these attempts to imitate the natural resistance of guinea-pigs by

asparaginase injections in mice is explicable on consideration of the shorter time available, between infection and death, for the development of effective immunity in mice. Accumulated data over many years give values of 3.8 and 9.5 days for the mean time to death of mice and guinea-pigs respectively from intraperitoneal doses of 10^3 MP6 organisms. Doubling of these times for Exu infections (the growth rate of Exu organisms is about one-half of that of MP6 in asparagine-free blood) would give more than sufficient time for guinea-pigs but less than sufficient for the development of immunity in mice.

It is unfortunate that isolates from elsewhere in South America are not available for comparison with the Exu isolates (M. Baltazard, personal communication) to determine the generality of semi-dependence on asparagine for growth. If the Exu strains are typical, the restriction of the *Cavioidea* to S. America may in part be because of their unique immunity to the resident strains of *P. pestis*. The Exu isolates provide an example of host-specificity in a bacterial pathogen being determined by its nutritional requirement and the nutritional adequacy of the different host species. Further, they serve to warn that a demonstration of non-pathogenicity of a bacterial strain toward a presumed sensitive animal species need not assure its safety for man.

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Effect of ACTH on Extinction of Rewarded Behaviour is blocked by Previous Administration of ACTH

THE behavioural effects of "frustrative nonreward"¹ (that is, the omission of an expected reward) seem to be particularly sensitive to certain drugs, notably sodium amylobarbitone and alcohol^{2–4}. A study of the effects of adrenocorticotrophic hormone (ACTH) on this kind of behaviour began recently in this institute. This hormone, although it is quite different pharmacologically from amylobarbitone and alcohol, turns out to resemble them^{5–8} in blocking two complex behavioural consequences of frustrative nonreward: the partial reinforcement acquisition^{9–11} and extinction¹² effects. The two partial reinforcement effects are observed when an animal's response is rewarded or not rewarded in an unpredictable manner; both were blocked by 8 IU of ACTH injected during training only¹³.

I therefore decided to investigate the effects of ACTH on a simpler consequence of frustrative nonreward—the extinction of a rewarded response. The experiment was designed* so as to contain four groups of eight rats: two of these received an injection of ACTH on day 1 of acquisition; the others received the gel placebo. One of each of these pairs of groups received ACTH injections during extinction, and the other received placebo injections.

Subjects were thirty-two male Wistar albino rats of mean weight 320 g. Throughout the experiment temperature was maintained as near as possible to 20° C. The apparatus consisted of a straight wooden alleyway, described in detail by

* This design was used to compensate for an initial mistake: some rats were injected with ACTH instead of the intended placebo on the first day of acquisition.

Gray⁵, divided by 'Perspex' guillotine hand-operated doors into an 18 cm startbox, a 164 cm runway and an 18 cm goalbox. The procedure for a trial was essentially as described by Gray⁵. Running time was measured over the runway section of the alleyway to the nearest 0.1 s by stopwatch. Animals were run in groups of four made up of one from each experimental condition. All rats were on a 22 h food-deprivation schedule, being fed not less than 1 h after the end of a day's experimentation. Reward consisted of four 54 mg MRC Diet 41B food pellets and animals were allowed 15 s in the goalbox to consume them. All rats were given their daily subcutaneous injection, either with ACTH (2 IU in 0.5 ml. gel) or placebo (0.5 ml. gel), at least 2 h before the start of the experimental session.

The experiment started with 4 days' pretraining, during which subjects were allowed to explore the apparatus freely with unlimited food in the goalbox after placebo injections. There followed 6 days of acquisition, with eight trials each day and an inter-trial interval of 4–5 min. The daily session for each group lasted about 0.5 h. Placebo injections continued throughout acquisition, except that the two "acquisition ACTH" groups were given ACTH on the first day of acquisition only. There was then a 3 day break in the experiment, during which the two "extinction ACTH" groups began to receive injections of ACTH and the other two groups continued to be injected with placebo. Finally, there were 6 days of extinction, with six trials per day. The "extinction ACTH" groups continued to receive a daily injection of ACTH and the other two groups received placebo. No food was ever in the goalbox, but otherwise all procedures were the same as during acquisition. If a rat failed to reach the goalbox in 120 s it was removed from the apparatus. After two consecutive such trials it was excluded from the experiment and given the notional score of 120 s for all subsequent trials. The mean daily running times were submitted to analysis of variance for acquisition and for extinction separately, except that the last day of acquisition was included with the extinction data to permit statistical evaluation of change during extinction from asymptotic performance in acquisition. Weights were recorded at regular intervals throughout the experiment, and showed no sign of change as a result of injection of ACTH.

During acquisition there were no differences between any of the four groups, and they were all running at virtually identical speeds by the end. The results in extinction are shown in Fig. 1. Analysis of variance showed that the principal effect of "extinction drug" was significant (F , 7.16; d.f. 1 and 24: $P < 0.025$), as was the interaction between "extinction drug" and "acquisition drug" (F , 4.98; d.f. 1 and 24: $P < 0.05$) and the second order interaction between these two variables and "days" (F , 3.01; d.f. 6 and 144; $P < 0.01$). In addition, the main effect of "days" was significant (F , 44.92; d.f. 6 and 144; $P < 0.001$) as was the first-order interaction between "days" and "extinction drug" (F , 2.96; d.f. 6 and 144; $P = 0.01$).

The interactions between "acquisition" and "extinction drugs" and between these variables and "days" were further analysed by t -tests based on the error variances from the analysis of variance. These showed that ACTH injected during extinction into animals previously given only placebo considerably retarded extinction (that is, increased running speed) and that this effect grew steadily greater as extinction progressed ($P < 0.001$ for day 3 of extinction onwards). The two groups given the single dose of ACTH on the first day of acquisition, and then given placebo and ACTH, respectively, during extinction, in contrast, did not differ from each other on any day nor when the means across days were compared. Comparing the two groups given placebo during extinction, there was an effect of the acquisition drug treatment on day 6 of extinction only, when the group given ACTH on the first day of acquisition ran faster ($P < 0.01$). When the two groups given ACTH during extinction were compared, the effect of acquisition drug treatment was much greater: the group

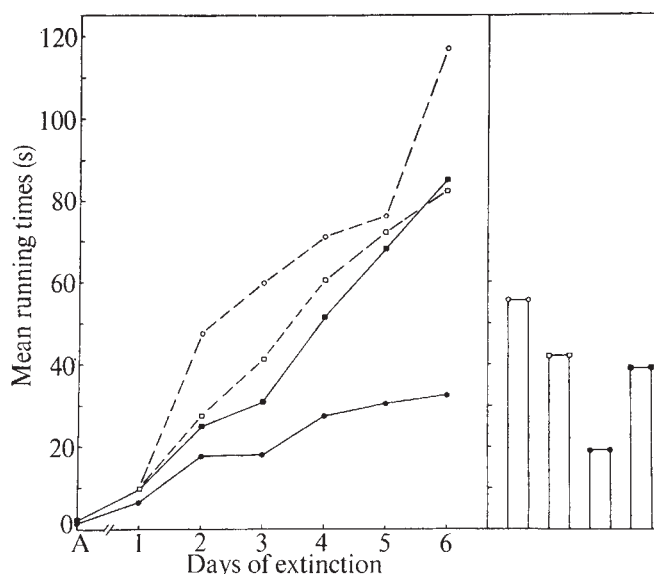


Fig. 1 Mean daily running time on the last day of acquisition (the point marked A on the abscissa) and on the 6 days of extinction as a function of acquisition and extinction injection of 2 IU of ACTH or placebo. The right-hand portion of the figure shows the means across days for the four groups. ○---○, Placebo on day 1 of acquisition and during extinction; □---□, 2 IU of ACTH on day 1 of acquisition, placebo during extinction; ●—●, placebo on day 1 of acquisition, 2 IU of ACTH during extinction; ■—■, 2 IU of ACTH on day 1 of acquisition and during extinction.

given ACTH in acquisition ran significantly more slowly from day 4 onwards, and the difference between the means across days for the two groups was also significant ($P < 0.02$).

The results of this experiment show that ACTH injected during extinction retards extinction, but that this effect is prevented by a single previous injection of ACTH on the first day of acquisition (6 days before the extinction drug treatment began). These results confirm those reported by Gray, Mayes and Wilson¹³ (who, however, required a larger dose—8 IU—to block the two partial reinforcement effects) in demonstrating that ACTH can block the behavioural effects of frustrative nonreward. The failure of ACTH injections during extinction to affect animals which had been given a single injection of this hormone 6 days previously is surprising. Whether prior administration of the drug alone is sufficient for this phenomenon, or whether exposure to the experimental situation in which extinction is subsequently carried out is also necessary, is not clear from our results.

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Incorporation of Uridine into Polysomes of Mouse Brain during Extinction

MICE injected with radioactive uridine in a double isotope labelling technique and then trained for 15 min to avoid a shock by jumping to a shelf incorporated about 50% more radioactivity into brain RNA¹ and polysomes² than untrained mice yoked to them. Quiet mice, yoked mice, and mice that were subjected to thirty electric shocks at random intervals for 15 min all incorporated less radioactive uridine into brain RNA and polysomes than did the trained mice^{1,2}. This indicates that the lights, buzzers, shocks, handling and activity have little effect.

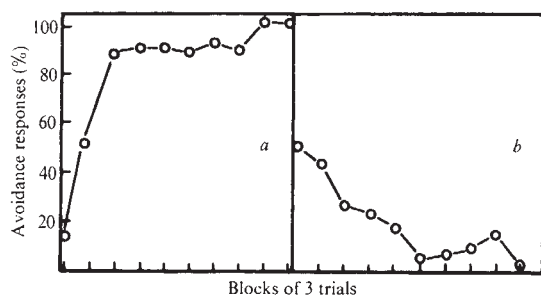


Fig. 1 Rate of acquisition and extinction of conditioned avoidance responses. Mice were trained or extinguished in the jump box apparatus in individual 15 min sessions. *a*, Avoidance training on the first day with no injection. The curve shows the average performance of 14 animals. *b*, The mice from *a* were trained on 3 consecutive days. *b* shows the performance curve for extinction of behaviour on the fourth day 30 min after an intracranial isotope injection (14 animals).

Here we report the effects of extinction training on the incorporation of uridine into brain polysomes. The training consisted of the one way jump box avoidance conditioning described previously^{1,3}. Briefly, mice were conditioned to avoid an electric shock by jumping to the shelf during a 3 second interval of light and buzzer presentation. The mice were given one 15 min session each day for 3 days. On the fourth day of training a naive mouse and a trained mouse were injected with radioactive uridine and after 30 min were placed in the training apparatus. The naive mouse was exposed to the yoke experience without shock. The light and buzzer were presented for 3 seconds, as during training, but the grid floor was never electrified. Approximately thirty presentations of the conditioned stimuli were given during the 15 min during which the animal eventually stopped jumping to the shelf. If the mouse in extinction training did not cease to respond to the conditioned stimulus after 10 min it was held on the floor for one trial (this applied to mouse pairs 247/248, 313/314 and 317/318).

The double isotope labelling method was used in these experiments¹. For each experiment two 6 to 8 weeks old male C57Bl/6J mice from the Jackson Laboratories, Bar Harbor, Maine, were used, one undergoing extinction and the other

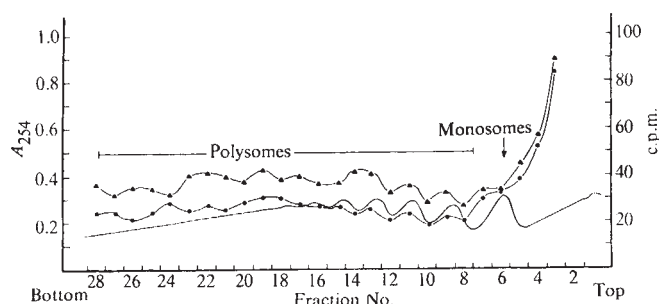


Fig. 2 Sucrose gradient centrifugation profiles of polysomes from mouse brain. One mouse was undergoing extinction while the other was serving as a yoked control. Procedures are as described in the text. Profiles shown are for pair 253 and 254 (Table 1). —, Absorbance at 254 nm; ●, ³H c.p.m., yoked mouse; ▲, ¹⁴C c.p.m., mouse undergoing extinction.

a yoked control. One mouse received 20 μ Ci of uridine-5-³H (Nuclear Chicago, s.a. 2 Ci/mmol, conc. 1 mCi/ml.) and the other received 4 μ Ci uridine-2-¹⁴C (Nuclear Chicago, s.a. 54 mCi/mmol, conc. 200 μ Ci/ml.) injected bilaterally into the lateral ventricles⁴. Both mice were killed immediately after the 15 min session and their brains were removed. An extra non-radioactive brain was added to the two radioactive brains as carrier. All subsequent procedures were carried out at 4° C. The brains were homogenized in 7 ml. of a solution containing 0.25 M sucrose, 0.005 M magnesium acetate, 0.024 M KCl, and 0.05 M Tris (hydroxymethyl) aminomethane (Tris) (pH 7.6). The homogenate was centrifuged at 900g for 10 min and then at 15,000g for a further 10 min. The supernatant suspension was collected and added to one ninth volume of 5% sodium deoxycholate in 0.03 M Tris (pH 8.2). The solution was shaken briefly, and after 10 min was layered on a discontinuous sucrose gradient composed of 1.5 ml. of 1.6 M sucrose, 0.07 M KCl, 0.004 M magnesium acetate, and 0.05 M Tris (pH 7.6), over 3.0 ml. of a 2.0 M sucrose solution in the same buffer. The gradient was centrifuged at 104,000g for 2.5 h in a Spinco SW 25.1 rotor. One ml. fractions were collected and analysed for absorbance at 254 nm and radioactivity. The methods of UMP pool determination have been described elsewhere^{1,2}.

Acquisition and extinction curves for the mice are shown in Fig. 1. After 15 min, extinction of the conditioned avoidance behaviour was complete for most animals (Fig. 1b), and these showed increased incorporation of radioactive uridine into brain polysomes (Fig. 2). The mean percentage increase in fourteen of these mice was 37% ($P < 0.001$) when compared with yoked mice. This compares favourably with the 46% increase in incorporation of uridine into brain polysomes when naive mice are trained². In pair 313/314, in which there was no chemical difference between the two mice, no extinction was observed either (Table 1).

We can now relate these data to previous studies which showed that previously trained mice which only performed the task for 15 min showed increased incorporation of RNA precursors into brain RNA and polysomes⁵. This difference in chemical response could be because of habituation to the training apparatus, or absence of the stress and pain associated with the foot shock, for these mice received very few shocks. Because the mouse undergoing extinction showed increased incorporation in spite of the fact that it had habituated to the apparatus and received no shocks, we conclude these variables are not important. Further, although there may be stress in the extinction situation, the animal is not subjected to pain,

and no organized locomotion is required for the extinction process. In fact the mouse learns not to perform and therefore correctly responds when he does not jump. No freezing behaviour was observed in any mice.

In our previous experiments increased incorporation of uridine into RNA occurred only when animals were trained in a conditioned avoidance situation⁵. The fact that animals which extinguish this behaviour show a similar biochemical response may indicate that similar processes are involved in both cases. Although it is possible that the biochemical response is directly related to learning or memory consolidation, alternative explanations involving non-specific stimuli and restorative roles for macromolecules cannot be ruled out.

Table 1 Incorporation of Radioactive Uridine into Polysomes of Brains of Mice Undergoing Extinction of the Conditioned Avoidance Response

Pair	Isotope in the mouse undergoing extinction	Ratio of radioactivity in UMP from mice undergoing extinction and yoked mice	Ratio of radioactivity in polysomes from mice undergoing extinction and yoked mice	% increase in radioactivity in polysomes of mouse*† undergoing extinction
245/246	¹⁴ C	1.82	1.91	+4.5
247/248	³ H	0.82	0.86	+5.0
249/250	¹⁴ C	3.75	4.01	+7.5
251/252	³ H	1.15	1.53	+33.1
253/254	¹⁴ C	0.89	1.66	+86.5
255/256	³ H	0.77	1.72	+123.3
309/310	¹⁴ C	1.02	1.14	+11.2
311†/312	³ H	1.85	1.47	-20.5
313/314	¹⁴ C	0.45	0.45	0.0‡
315/316	¹⁴ C	0.30	0.81	+170.0
317/318	³ H	1.35	2.02	+50.0
561/562	³ H	0.25	0.28	+12.0
563/564	¹⁴ C	0.79	0.93	+11.8
565/566	³ H	0.35	0.45	+28.5

* The mean per cent increase for these 14 pairs is +37.4%. The Wilcoxon test shows $P < 0.001$.

† The per cent increase is corrected for variations of radioactivity in UMP. The values are calculated as follows: $100[(P/U) - 1]$ where P = ratio of radioactivity in polysomes (mice undergoing extinction : yoke) and U = ratio of radioactivity in UMP (mice undergoing extinction : yoke).

‡ This animal did not extinguish the response and continued to jump to the shelf for 15 min.

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Olfactory Imprinting in a Precocial Mammal

WE wish to describe a new approach to the study of imprinting in mammals. As far as precocial mammals are concerned, only the reverse phenomenon of "maternal imprinting" on the neonatal young has been repeatedly investigated in sheep and goats¹⁻⁴. Shipley⁵ "imprinted" guinea-pigs to a wooden block and showed this to be a gradual learning process.

Early olfactory stimulation influences social behaviour in altricial mammals. Marr and Gardner⁶ sprayed pups of laboratory rats with cologne and found that they later preferred cologne-smelling rats, while pups treated with methyl-salicylate became socially anosmic as adults. Mainardi *et al.*⁷ treated parent mice with perfume, which led to the loss of sex discrimination by their young when adult. Fox and Himwich⁸ applied aniseed oil to the mammae of lactating bitches whose pups later responded positively to aniseed oil.

During studies of olfactory communication in black-tailed deer (*Odocoileus hemionus columbianus*)⁹⁻¹⁴ we designed an experiment to investigate olfactory imprinting. A fawn born in captivity was separated from its mother and sibling 1 h after birth. It received its first meal (colostrum) from its mother and was licked dry by her. The other sibling was left with the doe. The experimental animal was kept for 15 days alone in a pen, because the social bond between mother and fawn develops gradually during the first 2 weeks of life. A rack with four legs and wheels served as mother surrogate (Fig. 1). Attached to the apparatus was a milk bottle and a buzzer, which could be activated by a push button on the 3 m long handle, and which imitated the "low bleat" of a deer doe. The board at the rear of the surrogate had a scented filter paper on each side. Fresh scent was applied to this paper twice a day. The imprinting stimulus used was pronghorn or deer scent (about 100 µg of the principal component and other components in proportion, dissolved in 1 ml. of petroleum ether).

During the first day, whenever the fawn bleated, the experimenter outside the pen, hidden behind burlap, moved the "doe" slowly and activated the buzzer. The fawn moved toward the "doe" and sucked the bottle. During nursing the experimenter rubbed the fawn's anal area with a paper towel on a 2 m long stick to release elimination and stimulate further sucking.

Of four fawns, a male accepted a dummy with ischiadic scent of the male pronghorn (*Antilocapra americana*). Three other fawns (one male and two female) responded socially toward their "does", but did not suck efficiently and were removed from the experiment when the two males were 4 days old and the female was 16 days old.

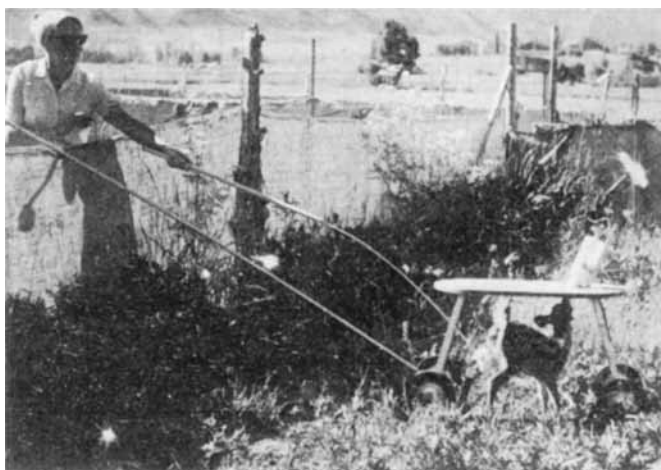


Fig. 1 Male black-tailed deer fawn, 22 days old, suckling at its surrogate mother. Simultaneously its anal area is rubbed with a paper towel attached to the end of a stick. This treatment simulates the licking by the natural mother.

During the first 2 weeks the mother surrogate became a social focal point for the fawn, and not merely a food source. When approaching the "doe" the fawn jerked its head and wagged its tail as fawns do to their mothers. It did not bleat in the presence of the "doe", but only when hungry and if the milk bottle was empty. When a person entered the pen, or a thunderstorm started, the fawn fled toward the "doe" and stayed close to it. During darkness, the fawn ate more pellets and pieces of apple near the "doe" than near the water ($P < 0.05$; Wald-Wolfowitz runs test).

When it was 15 days old the fawn was confronted ten times with two identical mother surrogates after its own "doe" had been removed. One dummy carried pronghorn scent, the other tarsal scent of black-tail or mule deer (*Odocoileus hemionus hemionus*) during alternate trials. The dummies stood parallel and 3–4 m from each other at varying places in the pen. If the fawn did not respond, the surrogates were moved and the buzzers sounded. This released new interest and new approaches.

Two observers outside the pen recorded the time spent by the animal 0.5 m or closer to each dummy, and the time spent near the filter papers (rear end). The fawn spent more total time and longer single time periods at the "pronghorn dummy" than near the other two (Table 1).

Table 1 Total Time Spent by a Male Black-tailed Deer Fawn with Three Different Surrogate Mothers in Twenty Choice Experiments after 2 Weeks of Exposure to Pronghorn Ischiadic Scent

	Test at 15 days of age				Test at 33 days of age			
	P	B	P	M	P	B	P	M
Total observation time (min)	98	98	95	95	100	100	100	100
Total time (s) spent near "doe"	364.2	179.4	311.2	266.8	364.5	72.6	123.8	173.4
Significance of difference* (P)	NS		NS		NS		NS	
Total time (s) spent near rear end of "doe"	321.4	75.8	273.6	92.1	314.2	51.3	118.7	59.5
Significance of difference (P)	0.025		0.01		0.028		NS	

* Mann-Whitney U Test. P, Pronghorn (male ischiadic scent); B, black-tailed deer (female tarsal scent); M, mule deer (female tarsal scent).

The animal was then left for 2 weeks with an unscented dummy. At 33 days of age, it was tested again in ten trials of 20 min each. After the "maternal scent" had been omitted for 2 weeks, the discrimination was almost as good as before. In both tests longer single time periods were spent at the rear end of the "doe" with pronghorn scent ($P < 0.05$, χ^2 test).

When it was 2.5 months old, the fawn was penned with three females, one pronghorn and one each of black-tailed and mule deer. In 120 observations at 2 min intervals it stayed closer to the pronghorn than to either of the deer ($P < 0.01$; Kolmogorov-Smirnov and χ^2 tests).

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Turnover of Basic Protein of Rat Brain

EXPERIMENTAL allergic encephalomyelitis (EAE) is a demyelinating disease which is induced by challenging experimental animals with a basic protein of myelin^{1–6}. Myelin has long been presumed metabolically inert, but recent evidence indicates that there may be a turnover of some of the lipids^{7,8}. One study has indicated a moderate turnover of proteolipid but little turnover of basic protein⁹. The present studies were undertaken to determine whether the basic protein is metabolically dynamic or stable.

Basic protein was isolated from whole brain¹⁰ and from myelin purified with Ficoll and sucrose gradients from rats receiving ³H-leucine. This protein produced clinical signs of EAE in three of four Lewis rats when it was injected in the foot pad at a concentration of 50 µg in Freund's complete adjuvant. Lewis rats (Microbiologicals) were injected subcutaneously four times at 12 h intervals in the dorsum of the neck with 0.5 µCi/g body weight of L-³H-4,5-leucine (20 Ci/mmol, Nuclear Chicago) beginning at age 10 days (immature) and 70 days (mature). By this method label was incorporated in animals which were rapidly forming myelin and in animals which are presumably slowly forming myelin. At regularly spaced intervals beginning at one week and

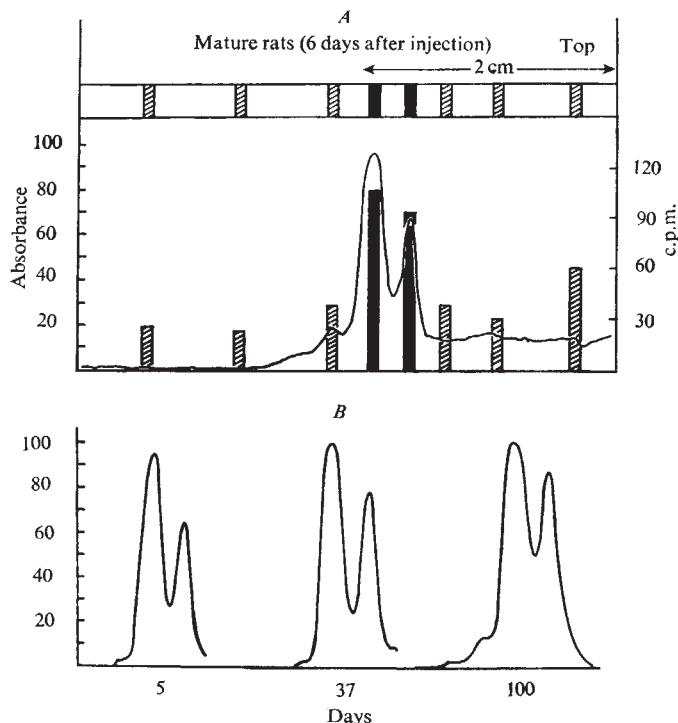


Fig. 1 A, Electrophoresis of 250 µg of sample for 5 h at 3 mA/gel. The black areas are the bands and the six hatched areas are the unstained slices from the gel. The bars represent c.p.m. and the solid line is absorbance. B, Density tracings from a series of disc gels from mature animals.

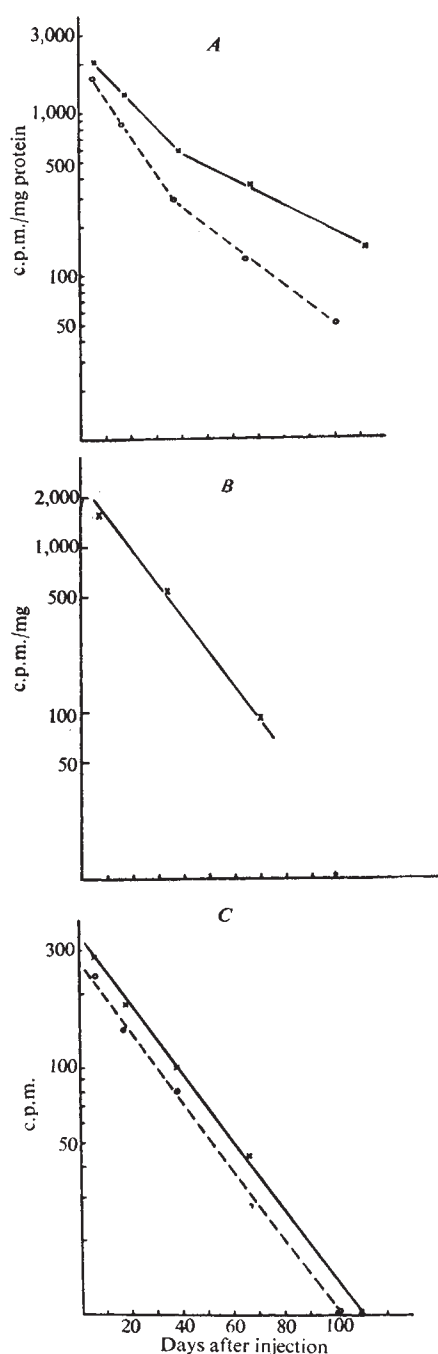


Fig. 2 *A*, Time course of loss of label from the basic protein isolated from whole brain. *B*, Loss of label from the basic protein isolated from myelin from mature animals. The myelin was washed twice with acetone, 1.5 ml./10 mg, and once with water, 0.5 ml./10 mg, before HCl extraction. *C*, Time course of loss of label from the basic protein purified by electrophoresis. $\times$ — $\times$, Mature; $\circ$ — $\circ$, immature.

extending to 4 months post injection, four to six rats were decapitated; their brains were pooled and stripped of their meninges. The basic protein was isolated by the procedure of Kies *et al.*¹⁰. After lyophilization, 2 mg samples were counted by liquid scintillation. In addition, at the same intervals the brains of three adult rats were homogenized in 20 ml. of 20% Ficoll in 0.15 M phosphate buffer (pH 7.4)–0.1 M KCl¹¹ and centrifuged at 6,000*g* for 40 min. The floating myelin was collected and washed twice with water. It was further purified by centrifugation for 2 h at 90,000*g* in a 12–30% sucrose gradient in an 'SW 25' Spinco rotor. The collected myelin fraction was washed with water three times, dialysed and lyophilized. Basic protein was isolated from the myelin by overnight extraction with dilute HCl (pH 3.0)

after two washes with acetone for 2 h and 20 min respectively and one wash with distilled water for 20 min.

The basic protein preparations from whole brain were purified by electrophoresis at pH 4.3 on 5 cm polyacrylamide disc gels¹². After electrophoresis the gels were stained with Buffalo black for 1 h and destained by electrophoresis. The rat basic protein was resolved into the two typical bands, previously described¹³, and the amount present was determined from the density of each band using a 'Photovolt' microdensitometer. The bands were cut from the gel together with strips from unstained areas of the gel for background (Fig. 1*A*) and dissolved in 0.25 ml. of 30% H₂O₂ to give a solution which was miscible with the counting solution¹⁴. The net counts were corrected for variations in the amount of protein in bands from different gels. Data from immature animals were corrected for the dilution of labelled myelin protein by the formation of new myelin as the animals matured. The correction factors were derived from data on the accumulation with age of cerebrosides and plasmalogens, lipids that are characteristic of myelin^{15,16}. No corrections were applied to the data from mature animals.

The loss of label from basic protein isolated from whole brains of immature and mature animals followed similar time courses (Fig. 2*A*). The curves indicated a considerable loss of label by 3 months after injection of isotope with only 5% of the radioactivity remaining in basic protein. The time course of loss of label from basic protein isolated from myelin is shown in Fig. 2*B*. Again, an almost complete loss of label from the basic protein was observed by 3 months. The label had completely disappeared by three months from the basic protein of both immature and mature animals purified by electrophoresis (Fig. 2*C*). There was no significant difference between the absorbance of the bands from mature rats of different ages (Fig. 1*B*). The density tracings from disc gels of protein from immature animals were also comparable except for the first point (6 days after injection). The bands from this gel were not as sharp as those from other time points and stained with less intensity. Also the two major bands were each split into two bands. The half life of the encephalitogenic basic protein, as determined from the disc gel data, was about 21 days in both the immature and mature animals.

These studies suggest that the basic protein turns over at a more rapid rate than would be expected were myelin a metabolically inert structure. This does not necessarily contradict the findings that some components of myelin are stable. It is, however, one more piece of evidence that at least a part of this membrane is metabolically active. It seems reasonable on the basis of this finding to begin to think in terms of a unique location in myelin for the basic protein.

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Glycoproteins from Connective Tissue of Twins

EARLIER investigations on glycoproteins from connective tissue have been mainly concerned with their chemical characteristics¹⁻⁶, but very little is known about the biological function of this group of glycoproteins. Our previous observations⁷ demonstrated the presence of a family of glycoproteins in cardiovascular connective tissue which varied from individual to individual and suggested a genetic basis for their differences. We have isolated glycoproteins from aorta, skin and kidney of bovine, ovine, and human twins and analysed by gel electrophoresis to determine whether variations in glycoproteins occurred among identical and nonidentical twin pairs. Because connective tissue glycoproteins also show enzymatic activities like esterase and phosphatase⁸, zymograms for these two enzymes were also obtained on polyacrylamide gels. The glycoprotein samples were analysed further by immunoelectrophoresis because they are highly antigenic³⁻⁷.

Aorta, kidney and skin tissues were collected from six bovine twin sets (two identical and four nonidentical) and from two pairs of ovine nonidentical twins immediately after the animals were sacrificed. The ages of these twin sets varied from two weeks to a year. Tissues from six pairs of human twins, two identical and four nonidentical (all immature infants, gestation period 5-6 months), were obtained from local hospitals immediately after autopsies. All the tissues were frozen at -40°C until used for extraction of glycoproteins.

Glycoproteins were isolated from the tissues by methods previously described³, which involved extraction of the tissue with 0.15 M NaCl and precipitation of the glycoproteins from the extract at 60-100% saturation of $(\text{NH}_4)_2\text{SO}_4$. The glycoprotein sample from each tissue was analysed by electrophoresis in polyacrylamide gel in a discontinuous buffer system⁹.

Electrophoresis of the samples containing 700-750 μg of glycoprotein was performed in an 'EC 470' vertical gel electrophoresis cell (E-C Apparatus, Philadelphia). A 4% 'Cyanogum-41' in Tris-HCl buffer (pH 6.7) was used as a spacer gel. The running gel consisted of 7% monomer in Tris-EDTA-boric acid buffer (pH 8.9). The same buffer system at pH 8.4 was used as the electrode buffer. The voltage was maintained at 200 V for 30 min and then increased to 300 V for the next 2 h. The gels were stained with amidoblack, periodic acid-Schiff (PAS) reagent⁷, or with specific stains for enzymatic activity^{8,10}. Glycoproteins from human twins were stained only for protein patterns (amidoblack) because of the limited availability of samples. Glycoproteins were further characterized by immunoelectrophoresis in agar^{7,11}. Although antisera to each organ studied could not be prepared, rabbit antisera to glycoprotein extracts from pooled bovine aortas were used in an attempt to characterize the glycoproteins.

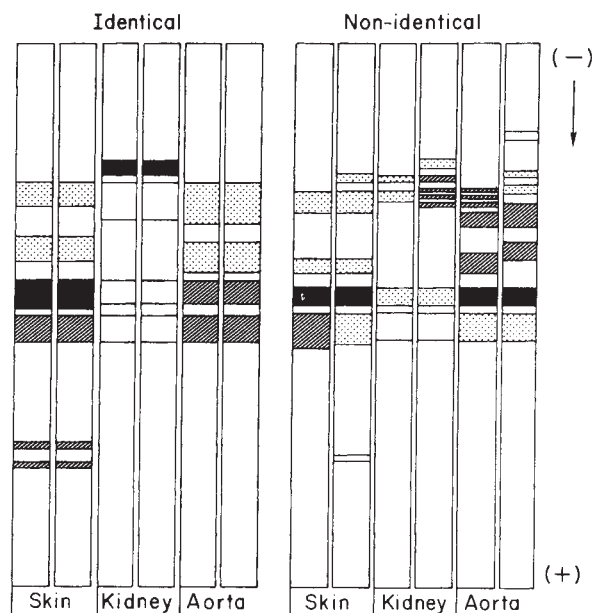


Fig. 1 Diagrammatic reproduction of polyacrylamide gel electrophoretic pattern of glycoproteins from skin, kidney and aorta of bovine twins. Electrophoresis was carried out using discontinuous gel electrophoresis technique. The gels were stained by PAS. The varying shades of the bands represent the difference in intensity produced by PAS. The dissimilarity in the pattern of glycoproteins from nonidentical twins as compared with the similarity in identical twins is apparent.

Several protein bands were observed in all samples after electrophoresis when the gels were stained with amidoblack. Similarly, the protein bands were detectable by PAS stain, indicating the presence of both protein and carbohydrate in the components being studied (Fig. 1).

All the identical twin pairs studied showed exactly similar electrophoretic patterns; but glycoproteins from different organs, skin, aorta and kidney, of the same identical twins showed considerable variations in the electrophoretic pattern for each tissue, indicating an organ specificity of these materials⁷. Furthermore, within the same species the electrophoretic pattern of one set of identical twins was found to be different from another set, which agrees with the concept of an individuality of glycoproteins⁷. Similar results were obtained with enzyme stains for esterases and acid phosphatases in the case of identical twins.

Glycoproteins from aorta, kidney and skin from the non-identical twins, except the kidney and skin of one bovine set, showed dissimilar electrophoretic patterns when stained with amidoblack or PAS. But zymograms of nonidentical bovine and ovine twin glycoproteins showed, in most cases, quantitative rather than qualitative differences. This is in agreement with the earlier observation of Arfors *et al.*¹², who observed that only about 35% of the dizygotic human twins showed discordant zymograms for serum phosphatases. The phosphatases are glycoproteins of tissue origin and are under genetic control like other isoenzymes¹³. Quantitative differences would also be in keeping with the observation of variations of enzyme levels occurring in different clones of cells in the heterozygous state¹⁴.

An example of immunoelectrophoretic patterns of the glycoproteins from aorta, kidney and skin of bovine identical and nonidentical twins is shown in Fig. 2. Although immunoelectrophoretic patterns of the glycoproteins from identical bovine twin pairs were similar, individual differences could be detected in the nonidentical twins. It is interesting that rabbit antiovine aorta glycoprotein gave precipitin bands against bovine kidney and skin glycoproteins, indicating the occurrence of proteins with common antigenic determinants in the organs studied. Similar cross-reactions were observed

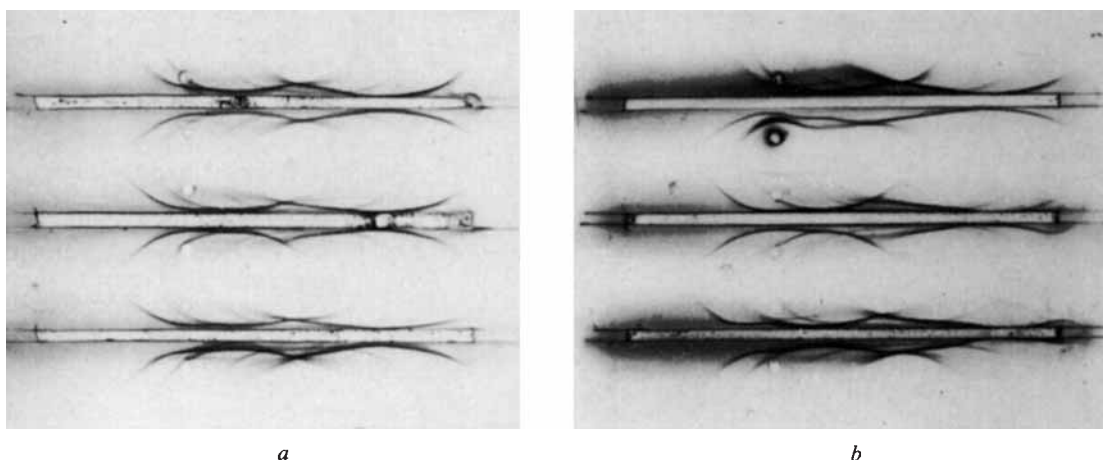


Fig. 2 Immunoelectrophoresis in agar of glycoproteins from aorta (upper), kidney (middle) and skin (lower) of bovine twins: *a*, identical; *b*, nonidentical. The precipitin bands were developed with rabbit antisera to glycoproteins from pooled bovine aortas (in trough).

with antisera to human sera with glycoproteins from the aorta. Because isolation and characterization of glycoproteins from connective tissue are only recent, very little is known at this time of their immunologic character.

Our earlier observation⁷ that the glycoproteins in connective tissue possess macromolecular individuality is further substantiated by the present investigation. The occurrence of identical glycoproteins in connective tissues of monozygotic twins but not in dizygotic twins again suggests a genetic basis in the make-up of these compounds. Furthermore, a role for glycoproteins in transplantation rejection is suggested by the studies of Castermans¹⁵, and more recently, murine histocompatibility antigens were shown to be glycoprotein in nature¹⁶. Other connective tissue compounds (collagen and mucopolysaccharides) have not shown such individual variations.

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Ultrastructural Localization of Pemphigus Autoantibodies within the Epidermis

INDIRECT immunofluorescent techniques show that the sera of patients with pemphigus vulgaris contain immunoglobulins (IgG) which specifically bind to the presumed site of the intercellular space of stratified squamous epithelia¹⁻³. Pre-titres of these diagnostic antibodies reflect the progress of the disease as well as the patient's response to treatment⁴. There is considerable evidence that the IgG immunoglobulins^{1,5} and complement⁶ found in the intercellular spaces represent autoantibodies⁴. These data imply that the distribution of the IgG deposits corresponds to the primary lesion in this disease^{7,8}.

The resolution of immunofluorescent microscopy, however, is not sufficient to establish whether the immunoglobulins are deposited within the intercellular space proper, on or in the cytomembranes, or in the peripheral cytoplasm of epidermal cells. Combined electron microscopic and immunocytochemical investigations were therefore undertaken. We now report the first data on the actual fine structural localization of immunoglobulins within pemphigus epidermis.

Our direct immunocytochemical method makes use of an enzyme-labelled anti-human globulin antibody to reveal the site of human immunoglobulins within the tissue. Concentrated anti-human globulin antibody (obtained from Progressive Laboratories, Baltimore, Lot 2827) was conjugated with highly purified horseradish peroxidase (obtained from Schuchardt, Munich, Lot PE 029) according to the method described by Avrameas⁹, using glutaraldehyde as a coupling agent. The skin of a 61 year old female patient with active pemphigus vulgaris was investigated. Samples of epidermis were removed from clinically normal and erythematous skin sites within the vicinity of bullous lesions by sliding pressure from a finger; the disease makes the skin fragile and easy to dislodge in this way (Nikolski phenomenon). The epidermal sheets were washed in phosphate buffered saline for 3 h;

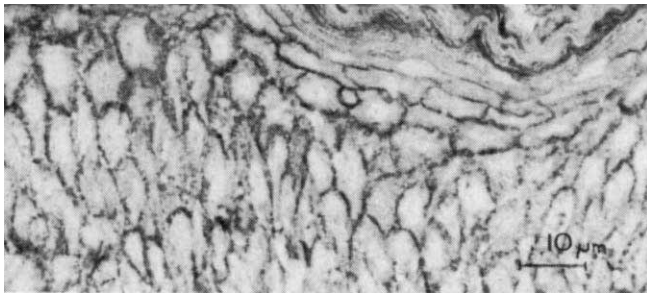


Fig. 1 The distribution of the anti-human globulin-peroxidase conjugate within the epidermis of a patient with pemphigus vulgaris, as seen with the light microscope. The reaction product is localized at the approximate site of the intercellular spaces. This staining pattern is identical to that obtained with fluorescein-labelled anti-human globulin.

40 μm sections were obtained with an S & F tissue sectioner and incubated in the conjugate diluted 1 : 10 with 0.1 M phosphate buffer (pH 7.2) for 12 h at room temperature. They were then washed in phosphate buffered saline and incubated in a cytochemical medium to reveal the sites of peroxidase activity¹⁰. After a rinse in phosphate buffered saline the sections were treated with osmium tetroxide and processed for electron microscopy.

As controls, some sections (a) were incubated in phosphate buffered saline containing 12.5 mg/ml. horseradish peroxidase, rinsed and reacted for peroxidase activity. (b) Others were incubated in non-conjugated anti-human globulin (15 mg/ml.) for 9 h (blocking reaction), washed in phosphate buffered saline, incubated in the conjugate, and after rinsing reacted for peroxidase activity. (c) Sections of shave biopsies obtained from the skin of normal individuals were incubated in the conjugate and processed as described. (d) For light microscopy, cryostat sections of the epidermal specimens were incubated with the conjugate at room temperature (3 h), washed in phosphate buffered saline and reacted for peroxidase activity. Light microscopic controls included incubation in a peroxidase solution and preincubation with non-conjugated anti-human globulin as described for the electron microscopic procedure. (e) Cryostat sections were also treated according to the conventional direct immunofluorescent procedure using fluorescein conjugated anti-human globulin (obtained from Progressive Laboratories, Baltimore) and were examined with a fluorescent microscope.

The reaction product of the cytochemical peroxidase procedure appears as dark brown precipitate in light microscopic sections and can be recognized in electron microscopic specimens by its high electron density. Previous studies^{11,12} had shown that this method fails to reveal any endogenous peroxidase activity within the epidermis and it was therefore concluded that any reaction product discernible in our material should indicate the site of the antibody-peroxidase conjugate. At the light microscopic level the reaction product was present throughout the epidermis and was localized in the region of the intercellular spaces (Fig. 1). Isolated acantholytic cells (cells separated because of the loss of epidermal coherence which characterizes the disease) were surrounded by a narrow rim of reaction product. The observed staining pattern was identical with the pattern of specific fluorescence¹ in the sections which had been treated with the fluorescein-anti-human globulin conjugate. These findings show that identical antigenic sites were visualized by the two different types of conjugates and techniques.

At the ultrastructural level electron dense material was regularly observed and was always confined to the intercellular spaces (Fig. 2A). The material was globular in appearance and never extended beyond the cell membrane into the cytoplasm of the keratinocytes. It was also observed within the extracellular portion of desmosomes but not all attachment devices contained the immunoglobulin. Widened inter-

cellular spaces contained no reaction product but electron dense material was seen coating the cell surfaces (Fig. 2A). Acantholytic cells also exhibited a thick layer of electron dense material resting on the outer lamina of their cytomembranes (Fig. 2B).

Control specimens which had been preincubated in non-conjugated anti-human globulin before treatment with the conjugate showed no intercellular staining patterns under either light or electron microscopy. Intercellular staining was also absent from tissue specimens incubated in the peroxidase solution and from specimens of normal skin treated with the conjugate. These controls rule out nonspecific trapping of the immunologically active tracer or of non-conjugated peroxidase within the intercellular spaces. The specific blocking of the reaction by the non-conjugated antibody suggested that the reaction product observed in the main experiment indeed indicated the site of immunoglobulins to which the anti-human antibody-enzyme conjugate had been bound.

Our observations show that the autoantibodies of pemphigus are deposited in and confined to the intercellular spaces of the epidermis. This agrees with the findings of Chorzelski *et al.*¹³ who demonstrated that ferritin-labelled globulins of pemphigus serum are bound to the intercellular compartment of rabbit oral mucosal epithelium. Epidermal cells have been shown¹⁴ to be coated by a glycocalyx and the glycocalyxes of adjacent cells constitute the "intercellular cement" or intercellular substance of the epidermis. It was postulated that the intercellular substance acts as a carrier for or contains the antigen to which the circulating antibodies of patients with pemphigus are directed. Our findings suggest very strongly that pemphigus antibodies are directed to this extracellular material.

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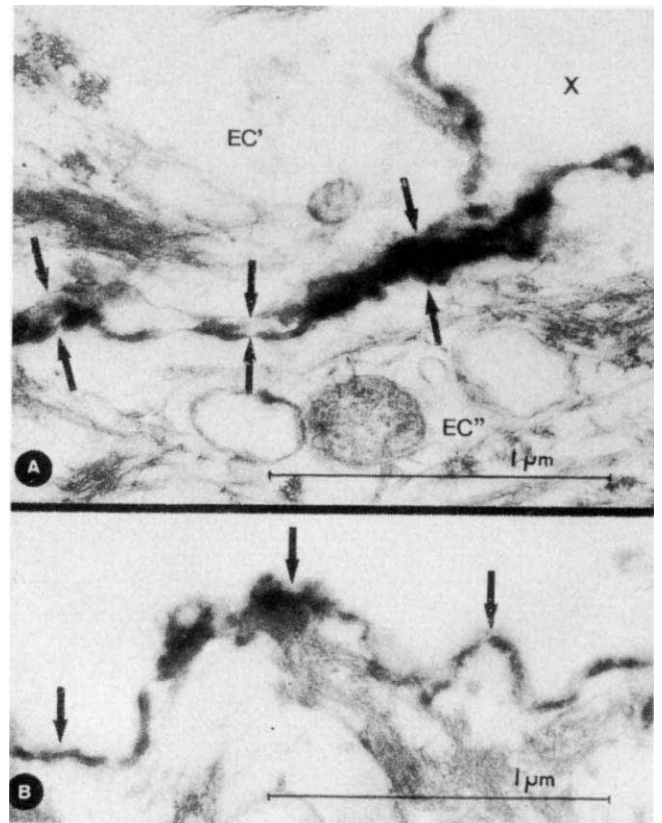


Fig. 2 A, Ultrastructural localization of the antibody-peroxidase conjugate. The electron dense reaction product is confined to the intercellular space (arrows) between two adjacent epidermal cells (EC', EC''). It is also seen coating the cell surfaces in the area where the intercellular space is distended (X). B, Immunoglobulins (arrows) coating the surface of an acantholytic cell.

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Denervation in Murine Dystrophy

MUSCULAR dystrophy in the mouse has long been regarded as a primary myopathy¹⁻³, but many recent observations contradict this view. For example, reductions in the frequency of discharge⁴ and in the amplitude⁵ of miniature end-plate potentials have been reported, and McComas and Mrozek⁶ have shown that large numbers of muscle fibres in dystrophic tibialis anterior and gastrocnemius muscles are "functionally denervated". These observations prompted us to make a detailed study of motor unit populations in normal and dystrophic mouse muscles.

Homozygous normal and dystrophic mice of the Bar Harbor 129 strain were anaesthetized with urethane. Isometric contractions of tibialis anterior muscles were recorded using an 'RCA 5734' strain gauge connected to the tendon of insertion with silver wire. The compliance of the recording system was 0.41 mm/kg and the frequency response of the loaded strain gauge was 800 c.p.s. The entire hind quarters of the animal were immersed in liquid paraffin maintained at 37° C. Preparations were excited by stimulation of the sciatic nerve⁷. Contractions of the first four to six putative motor units were recorded on the screen of a 'Tektronix 564' storage oscilloscope, measured and photographed. The maximal muscle twitch was next recorded. From these results two estimates of the number of motor units were obtained for each muscle: maximal muscle twitch tension divided by the tension of the most excitable unit, and maximal muscle twitch tension divided by the mean tension of the series of units. A typical experiment is shown in Fig. 1 and the detailed results in Table 1. In both normal and dystrophic muscles, the estimates made by the two methods were closely similar, but the mean number of motor units in dystrophic muscles was significantly lower than in normal; in fact, in only one out of eleven dystrophic muscles was the estimated number of motor units within the normal range.

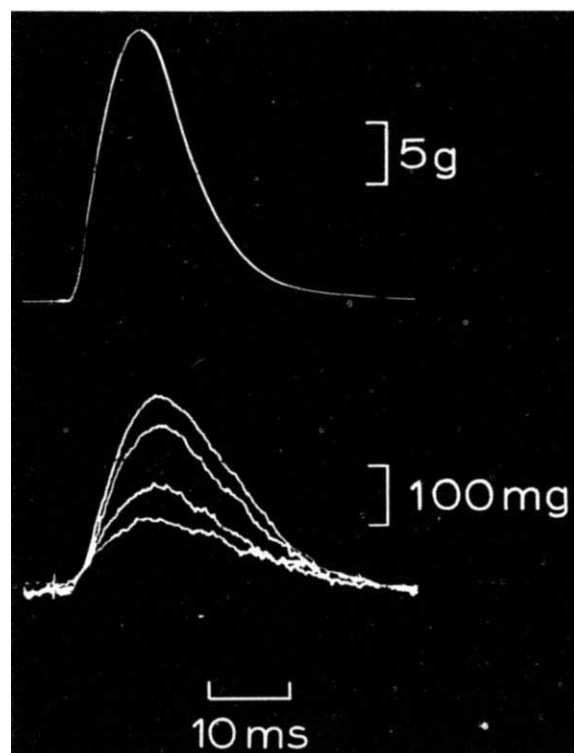


Fig. 1 Normal tibialis anterior. Upper trace: isometric maximal twitch response; lower trace: response of first four units of normal tibialis anterior muscle in 3 month old female Bar Harbor 129 strain mouse. Maximal twitch tension 20.2 g; tension of first unit 118 mg; mean tension of all units 82.75 mg. Motor unit estimate from first unit 171, and from mean tension of all units 244.

The mean twitch tension generated by dystrophic units was significantly reduced (normal, 130 ± 11.9 mg ($n=39$); dystrophic, 73.2 ± 5.5 mg ($n=44$), $P<0.01$), although most dystrophic units were within the normal range of amplitude.

Table 1 Estimated Numbers of Motor Units in Normal and Dystrophic Tibialis Anterior Muscles (Mean $\pm$ s.e. of Mean)

	Normal	Dystrophic	P
Motor unit estimate from first motor unit	223 ± 25.2 (10)	68 ± 13.1 (11)	<0.01
Motor unit estimate from mean amplitude of a series of units	214 ± 24.5 (10)	63 ± 11.0 (11)	<0.01
Mean tension of all recorded units (mg)	130.03 ± 11.88 (39)	73.2 ± 5.52 (44)	<0.01
Maximum muscle twitch tension (g)	26.43 ± 3.9 (10)	3.87 ± 0.84 (11)	<0.01

Values in parentheses represent number of observations. Where the variances were unequal, Welch's test was used to test the difference between means.

An attempt was made to correlate our physiological findings with anatomical evidence of denervation. Accordingly, tibialis anterior nerve-muscle preparations were dissected from normal and dystrophic mice and fixed for 4 h in Flemming's solution without acetic acid. After fixation, the preparations were embedded in paraffin, and sections 5 μ m thick were cut. The nerve branches supplying tibialis anterior were recognized as they left the muscle and followed almost as far as their junction with the principal nerve trunk. The sections were stained by the technique of Wiegert-Pal (Kultschitsky's modification), and the myelinated nerve fibres were counted and measured. We assume that 60% of the muscle nerves are efferent⁸ and that 60% of the efferent nerves are skeletomotor^{8,9}. On these

assumptions, the mean number ($\pm$ s.e.) of skeletomotor nerve fibres in normal nerves was 191 ± 26 ($n=6$, range 133–314), which closely agrees with our physiological findings; the mean number of estimated skeletomotor fibres in dystrophic nerves was 72 ± 14 ($n=4$, range 44–106), which is also in close agreement with physiological results. It is also of interest to note that the total number of myelinated nerve fibres in the dystrophic muscle nerves was so low compared with normal (dystrophic mean, 200 ± 41 , $n=4$; normal mean, 529 ± 72 , $n=6$) that the reduction cannot be accounted for on the basis of a reduction only in skeletomotor fibres. This suggests that there are abnormalities in the innervation of the sensory apparatus in dystrophic muscle that are worthy of further investigation.

There would seem to be two possible explanations for these results. Either the denervation observed is secondary to a random loss of muscle fibres from motor units, or the denervation is a primary event. The former possibility is unlikely because most of the dystrophic motor unit twitch tensions were within normal limits of amplitude. If the primary event had been in the muscle, one would expect normal numbers of very weak units. This was never observed. The second possibility is reasonable in view of the known trophic effects of nerve on muscle^{10,11}, since any disease of the motoneurone, however subtle, may be expected to be made manifest in the muscle. That many motor units are weak in dystrophy is not inconsistent with this view because a "sick" unit may be expected to become weaker before actually dropping out completely.

Our observations strongly suggest that denervation plays a considerable part in murine dystrophy. In this respect the results closely resemble those recently reported in dystrophia myotonia¹², Duchenne¹³ and limb girdle¹⁴ types of human dystrophy. The results imply that the primary abnormality in dystrophy involves motor nerves rather than muscle fibres.

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Nutrition and Aflatoxin Carcinogenesis

ALTHOUGH malnutrition and aflatoxins coexist in many areas of the world where there is a high incidence of liver disease and hepatocarcinoma, animal experiments so far have not precisely duplicated the disease syndrome and the morphological picture observed in man¹⁻⁸. We have examined interactions between nutritionally induced liver disease and experimental aflatoxin carcinogenesis and found that diets marginal in lipotropes result in remarkable differences in the response of rats to aflatoxin. This work was suggested by observations that diets low in protein had variable effects^{7,9} on aflatoxin toxicity and carcinogenicity. Furthermore, dietary lipotropes, especially methionine and vitamin B₁₂, may be critically low in many areas where liver carcinoma and mycotoxins coexist, and so we considered marginal lipotropes to be of more practical significance than low protein *per se* in the overall evaluation of interactions between nutrition and aflatoxin and their relation to carcinogenesis.

The experimental diet marginally deficient in lipotropes had 12% peanut meal, 3% casein and 6% gelatine as its protein source and is high in fat, 30% beef fat and 2% corn oil. The complete diet used as a control contained 23% casein and 16% corn oil. These two diets were used because of known effects on the liver rather than in an attempt to study specific dietary requirements. The deficiency does not result in cirrhosis even when experienced for long periods, although it doubles the lipid content of the liver⁸. When the stress of aflatoxin is superimposed on the marginal lipotrope deficiency, a marked difference from the normal response is observed. It is the interaction of diet and carcinogen that we consider important.

Male rats, Charles River Sprague-Dawley CD strain and Fischer strain, were given the control diet for 1 week after weaning, and then some of them received the marginal lipotrope diet. For acute toxicity studies, the respective diets were offered for 2 weeks *ad libitum*, after the 1 week conditioning period, and the rats were then given a single dose of aflatoxin B₁ (AFB₁) dissolved in 0.1 ml. of dimethylsulphoxide. Table 1 shows characteristic results of the effect of diet on the acute toxicity of AFB₁. The marginal lipotrope deficiency protected rats against doses of AFB₁ which were lethal to 60–100% of rats fed the control diet. An effect of differences in gastrointestinal absorption or alteration of AFB₁ was precluded by the intraperitoneal route of administration; the marginal lipotrope group was still protected.

Table 1 Effect of Diet on Acute Toxicity of AFB₁

Strain and route	Diet	2 week mortality
		No. of rats dead
Sprague-Dawley Intragastric	Marginal lipotrope	No. treated 0/5
	Control	3/5
	Marginal lipotrope	0/5
Intraperitoneal	Control	5/5
Fischer Intragastric	Marginal lipotrope	0/10
	Control	10/10

All rats were given a single dose of 7 mg/kg aflatoxin B₁.

A striking and consistent histological change in the livers of marginal lipotrope groups after a carcinogenic dose of aflatoxin (fifteen daily doses of 25 μ g each) is the appearance of focal areas of abnormal hepatocytes which are hyperplastic as indicated by increased uptake of ³H-thymidine. We have described these areas before⁸ and regard them as preneoplastic changes. The early appearance of such lesions in animals fed on the marginal lipotrope diet correlates well with a short tumour induction time. Table 2 shows characteristic observations which indicate a close correlation of early appearance of liver cell tumours in marginal lipotrope

animals. Hyperplastic nodules were present in significant numbers immediately after the carcinogenic dose of AFB₁ in the marginal lipotrope group, but were not observed in the control group in significant numbers until 6 months after administration of AFB. Tumours were found from 6 months onwards in the deficient rats and not until 12 months in the controls.

Table 2 Marginal Lipotropes and AFB₁: Focal Parenchymal Hyperplasia and Tumour Incidence

Dietary treatment	Appearance of hyperplastic nodules after exposure to AFB ₁	Hepatocarcinomas (%) months after exposure to AFB ₁ *			
		6	9	12	18
Marginal lipotrope	1-2 days	25*	50*	60*	85*
Control	3 weeks-6 months	0	0	30	60

Transformation of chemical compounds by the liver has been associated with enzymes which metabolize drugs and so we have examined the effects of diet alone and of diet plus AFB₁ on three enzyme systems. Our results show that the marginal lipotrope diet alone decreases both the demethylating enzymes and the hydroxylating enzyme (Table 3); perhaps more important, the demethylating enzymes were not inducible by AFB₁ in the marginal lipotrope groups (Table 4). The hydroxylase was decreased by AFB₁ in control animals but it was unaffected in marginal lipotrope animals.

Table 3 Effect of Diet on Liver Enzymes

Enzyme	Marginal lipotrope	Control
Aminopyrine demethylase*	305±23	481±55
<i>p</i> -Nitroanisole demethylase†	536±116	1,132±74
Benzpyrene hydroxylase‡	99±11	184±16

* μg of aminoantipyrene/h $\pm$ s.e.

† μg of *p*-nitrophenol/h $\pm$ s.e.

‡ Quinine units (10) $\pm$ s.e.

Table 4 Effects of Diet and AFB₁ on Liver Enzymes

Enzyme and treatment	Marginal lipotrope	Control
Aminopyrine demethylase, control	220±13	284±6
Aminopyrine demethylase, AFB ₁	217±37	460±122
<i>p</i> -Nitroanisole demethylase, control	1,147±77	1,423±58
<i>p</i> -Nitroanisole demethylase, AFB ₁	1,274±79	1,684±34
Benzpyrene hydroxylase, control	36±9	147±15
Benzpyrene hydroxylase, AFB ₁	35±4	60±5

Units are the same as in Table 3, expressed per gram of dry, fat-free liver $\pm$ s.e. Rats were given five daily doses of 25 μg of AFB₁.

Several equally valid hypotheses can be constructed from these observations. Based on the correlation of decreased AFB₁ toxicity in marginal lipotrope groups with decreased enzyme activity, it seems reasonable to speculate that AFB₁, the parent compound, is not toxic but must be metabolized to a toxic product. Normal livers make this conversion easily, whereas livers of marginal lipotrope groups do not, or do so only after repeated doses of AFB₁. Studies of the metabolism of AFB₁ have recently been reviewed and extended to several species¹¹. Differences in the rate of metabolism of AFB₁ by liver microsomal enzymes in different species could not be consistently correlated with differences in susceptibility to the acute toxicity of AFB₁. It was shown that the nature of the metabolites produced varied with the species, although with one exception, aflatoxin M₁, the structure of the metabolites and their effects were unknown¹¹. Therefore, within a given species changes in the concentrations of microsomal enzymes may correlate with the production of a toxic or a nontoxic metabolite and therefore with the toxicity of a given dose of AFB₁.

The carcinogen may be the parent compound itself or an intermediate between the parent compound and the toxic

product. The early appearance of hyperplastic nodules, the decreased enzyme concentrations and the short tumour induction time in marginal lipotrope groups suggest that nutritional modification of AFB₁ action offers a promising means for further study of mechanisms of its carcinogenesis and is of particular significance in view of the high tumour incidence in areas in which malnutrition and AFB₁ food contamination occur.

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DDT Movement from Adipocyte to Muscle Cell during Lipid Utilization

STORAGE of organochlorine pesticides such as 1,1,1-trichloro-2,2-bis-chlorophenyl-ethane (DDT) in adipocyte lipid of avian¹⁻⁴ and other species⁵⁻⁹ suggests that this is an effective detoxifying mechanism for these lipophilic biocides. Adipose tissue storage, however, is non-permanent because the normally slow release of DDT and its metabolites into the circulating fluids is probably increased during periods of accelerated lipid mobilization induced by starvation or increased energy expenditure^{6,10,11}. Data in the literature demonstrate that starvation results in higher blood levels of DDT^{2,6,12}, changes in tissue concentrations^{2,10,12,13} and increased excretion^{2,6,14}. But neither the mobilization rate nor the fate of the mobilized DDT has been quantitatively defined in the existing literature.

Results presented in this report quantify the mobilization of DDT and metabolites from adipose tissue and the concurrent accumulation of DDT and metabolites in muscle tissue of pigeons subjected to starvation at 6° C.

Body burdens of DDT were established in *ad lib* fed homing pigeons (body weight range 330-460 g) by dosing with ¹⁴C-DDT (randomly ring labelled, New England Nuclear) dissolved in corn oil at concentrations of 0, 0.15, 1.5 or 16%. During a 16 day dosing period, four groups of twelve birds each were individually force-fed with two gelatine capsules per day, each capsule containing about 64 mg of the appropriate DDT-corn oil solution. This resulted in total ingested amounts of 0, 3.101, 32.07 or 335.0 mg of DDT per bird.

Ad lib feeding was continued for 3 days after dosing was completed and then six birds from each group were killed to determine the total body burden of DDT and metabolites. At the same time the remaining six birds in each group were individually caged in a 6° C room with water available, but no food. The birds were killed 5-7 days later when they had lost 18-20% of their pre-stress body weight,

a condition which corresponded to 50–75% utilization of total carcass lipid. This extent of lipid depletion does not impair the bird's ability to regain normal weight when food becomes available.

All birds were killed by decapitation, blood was collected in heparinized containers and plasma separated from erythrocytes. Carcasses were plucked, and then the brain, liver, heart, omental fat pad and 10 g of breast muscle were dissected out. The remaining carcass tissue was ground up by a Hobart food chopper and two 40 g samples were taken for analysis.

Total lipid was extracted from each tissue and carcass sample by the Bligh and Dyer¹⁵ procedure and the lipid content determined gravimetrically. The ¹⁴C content of the co-extracted DDT and its metabolites was measured by liquid scintillation spectrometry using a scintillation solution of 0.5% 2,5-diphenyloxazole in toluene. The DDT quantities to be reported represent the sum of DDT and all its metabolic products.

Analysis of tissues from the 32.07 mg dosed birds (Table 1) indicates that the starvation and cold did not alter the percentage lipid or net DDT content of erythrocytes, plasma, liver or brain. A decrease in the percentage of lipid occurred in heart muscle with no net change in DDT content. Both the lipid and DDT content of the omental fat pad were considerably decreased by the starvation stress. Concomitantly the lipid content of breast muscle did not change but its DDT content increased markedly. These data demonstrate that the principal starvation-induced changes in DDT content occur in adipocyte lipid and muscle tissue. These changes were also quantitatively measured in birds dosed with 3.101 mg and 335.0 mg DDT (Table 2).

During starvation at 6° C there was a considerable reduction

in lipid content of the omental fat pad (Table 2) for all dose groups. Lipid utilization was accompanied by a net decrease in the total amount of DDT in the fat pad, but there was a concomitant increase in concentration of DDT per g lipid, indicating that fatty acids were moving out of adipocytes more rapidly than DDT. Furthermore, data in Table 2 demonstrate that the lipid utilizing stress did not alter lipid content of breast muscle, but did result in a net movement of DDT into muscle for all three body burdens of DDT. The extent of this movement is exemplified by the marked increases in muscle concentration that occurred in all treatment groups.

The increase in DDT content and concentration per g lipid induced by starvation was found to be the same in leg muscle as in breast muscle. The leg muscles and breast muscles account for 80–90% of the total muscle mass, so the breast muscle values in Table 2 can be considered representative of total body muscle. Thus, by determining the net amount of DDT relocating in total muscle mass as well as the amount of DDT irreversibly cleared from the carcass, we can obtain a minimum quantitative estimate of DDT mobilization.

The muscle mass dissected from carcass was found to be 44% of the plucked carcass weight and therefore used with the Table 2 data to determine the DDT content of total carcass muscle for the birds at each dose level (Table 3). The increase in carcass muscle content of DDT that occurred during the starvation stress represents net DDT movement into carcass muscle. Total carcass content of DDT before and after stress was determined (Table 3) by summing up the amounts of DDT extracted from the tissues. The decrease in total carcass DDT, which resulted from the lipid utilizing stress, represents the amount of DDT that was irreversibly cleared from the system. When the amount of DDT moved into muscle is

Table 1 Lipid and DDT* Content of Pigeon Tissues

Tissue	Lipid † (%)		Total DDT ‡ (mg)		DDT concentration ‡ (µg/g lipid)	
	Unstressed	Stressed §	Unstressed	Stressed §	Unstressed	Stressed §
Erythrocytes	0.54	0.57	0.0056 ± 0.0005"	0.0062 ± 0.0001"	1,020 ± 24	1,132 ± 28
Plasma	1.19	1.32	0.020 ± 0.002"	0.020 ± 0.002"	1,684 ± 67	1,587 ± 104
Liver	4.27	4.98	0.601 ± 0.024	0.658 ± 0.042	1,995 ± 36	2,050 ± 35
Brain	9.78	9.51	0.086 ± 0.002	0.089 ± 0.003	407 ± 7	424 ± 7
Heart	5.79	3.98	0.296 ± 0.013	0.317 ± 0.010	1,173 ± 94	2,030 ± 89
Omental fat pad	85.9	42.3	1.81 ± 0.11	0.525 ± 0.081	833 ± 108	2,524 ± 282
Breast muscle (10 g)	3.33	3.30	0.385 ± 0.010	0.664 ± 0.073	1,156 ± 63	2,012 ± 118

* Sum of DDT and metabolites, for birds that received the 32.07 mg dose.

† Each value is an average from six birds.

‡ Each value is an average from six birds with standard error of the mean.

§ The stress consisted of starvation at 6° C until 50–75% of the total carcass lipid was utilized.

" Values are mg per g wet weight.

Table 2 Lipid and DDT* Content of Breast Muscle and Omental Fat Pad

Tissue	DDT dose (mg/bird)	Total lipid † (g)		Total DDT † (mg)		DDT concentration † (µg/g lipid)	
		Unstressed	Stressed ‡	Unstressed	Stressed ‡	Unstressed	Stressed ‡
Omental fat pad	0	2.218	0.487	0	0	0	0
		± 0.368	± 0.082				
	3.101	1.791	0.187	0.170	0.044	95	235
		± 0.236	± 0.107	± 0.012	± 0.015	± 18	± 113
	32.07	2.172	0.208	1.81	0.525	833	2,524
		± 0.400	± 0.065	± 0.11	± 0.081	± 108	± 282
Breast muscle (10 g)	0	2.607	1.085	14.4	12.1	5,523	11,152
		± 0.669	± 0.357	± 2.4	± 2.2	± 678	± 2,935
	3.101	0.303	0.321	0	0	0	0
		± 0.029	± 0.044				
	32.07	0.366	0.314	0.038	0.069	104	220
		± 0.026	± 0.074	± 0.002	± 0.008	± 7	± 42
	335.0	0.333	0.330	0.385	0.664	1,156	2,012
		± 0.022	± 0.039	± 0.010	± 0.073	± 63	± 118
	335.0	0.350	0.390	1.30	3.41	3,714	8,744
		± 0.042	± 0.027	± 0.22	± 0.40	± 312	± 1,453

* Sum of DDT and metabolites.

† Each value is an average from six birds with s.e.

‡ The stress consisted of starvation at 6° C until 50–75% of the total carcass lipid was utilized.

Table 3 DDT* Mobilization During Lipid Utilizing Stress†

DDT dose (mg/bird)	Treatment	DDT content of total muscle ‡ (mg)	Increase in DDT content of total muscle during stress (mg)	DDT in total carcass§ (mg)	Decrease in DDT content of total carcass (mg)	Quantity of DDT mobilized during stress¶ (mg)
3.101	Unstressed	0.54		3.210 **		
	Stressed	0.99	0.45	2.538	0.672	1.122
32.07	Unstressed	5.66		32.56**		
	Stressed	9.76	4.10	27.58	4.98	9.08
335.0	Unstressed	19.37		254.1 **		
	Stressed	50.81	31.44	243.0	11.1	42.54

* Sum of DDT and metabolites.

† The total carcass lipid quantities were: (a) pre-stress 42.90, 39.24, 51.36 g and (b) post-stress 10.81, 13.73, 24.96 g, respectively, for the groups dosed with 3.101, 32.07 or 335.0 mg of DDT.

‡ Total muscle quantities were 143, 147 and 149 g for the 3.101, 32.07 and 335.0 mg DDT dose groups, respectively.

§ Total DDT in carcass is summation of quantity extracted from all component tissues.

|| Quantity of DDT no longer present in total carcass tissues.

¶ Sum of DDT quantity moved into muscle and quantity no longer in the carcass.

** Total DDT in carcass of unstressed birds represents the pre-stress body burden.

added to this we have a minimum estimate of DDT mobilization from adipocyte lipid (Table 3). Thus, pigeons with DDT body burdens of 3.21, 32.56 and 254.1 mg were able to mobilize, respectively, 1.122, 9.08 and 42.54 mg of the DDT, and relocated 0.672, 4.98 and 11.1 mg of it in muscle tissue.

The portion of mobilized DDT which the birds were unable to clear irreversibly could have been redistributed into any tissue. But it was relocated only in muscle and not in brain, liver, heart or blood, and may therefore be an important means of protecting DDT-sensitive target tissues, such as the central nervous system. On the other hand, accumulation in muscle tissue could result in degenerative changes in muscle cells and peripheral regions of the nervous system. DDT associated with muscle tissue, however, may be relatively innocuous, for the DDT is probably located in the intracellular lipid droplets, which Grinyer and George¹⁶ have observed in red muscle cells.

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Evaporation of DDT

THE movement of unchanged DDT from treated crops into the atmosphere has been suggested to contribute to the widespread contamination of the environment by this substance¹. In field conditions, 50% of DDT applied to the surface of soil has been found to disappear in 16–20 weeks (ref. 2) and 60% of DDT applied to an apple orchard could not be accounted for³. Ward and Burt⁴ attributed losses of DDT from glass plates and leaf surfaces to evaporation, but little other work seems to have been published on this question.

The probable rate of loss of DDT can be calculated from the rate of evaporation, which is dependent on its vapour pressure, and from the rate of diffusion of the evaporated molecules through the layer of still-air above the deposit. At low rates of evaporation the requirements of latent heat will not disturb the thermal energy balance. The vapour pressure of DDT is 1.5×10^{-7} mm of mercury at 20° C (ref. 5), the gaseous diffusion coefficient is assumed to be $0.05 \text{ cm}^2 \text{ s}^{-1}$, and the still-air layer thickness 2 mm (ref. 6), to give a rate of evaporation of DDT of $3 \times 10^{-3} \mu\text{g cm}^{-2} \text{ h}^{-1}$ at 20° C; at 5° C, vapour pressure 1×10^{-8} mm the rate is $2 \times 10^{-4} \mu\text{g cm}^{-2} \text{ h}^{-1}$.

An alternative way of calculating the evaporation rate is to use the relation between the rate of loss (W) and the product of the vapour pressure (P) and the square root of the molecular weight (M), as given by Hartley⁷. By inserting the appropriate values for DDT into Hartley's Table 1, and using a mean value of 5.4 for the relationship $W/P\sqrt{M}$, the rate of evaporation of DDT in the conditions of his experiment is calculated to be $2 \times 10^{-3} \mu\text{g cm}^{-2} \text{ h}^{-1}$.

To determine the evaporation rate experimentally, DDT uniformly labelled with carbon-14 in the phenyl rings was applied in ethanol to aluminium planchets (3.8 cm^2) to give an initial deposit of $0.5 \mu\text{g cm}^{-2}$. Ten replicate planchets were radio-assayed by end-window Geiger-Müller tube in an automatic sample changer⁸ during 53 days in a well ventilated room away from direct sunshine. Counts obtained from the planchets decreased steadily from the initial value of about 180 c.p.s. to 10 c.p.s. after 53 days. The interior of the lead castle and Geiger-Müller tube assembly became contaminated, and background counts rose from 0.2 c.p.s. to more than 20 c.p.s. during 36 h, but the apparatus was decontaminated five times during the experiment. In another experiment, planchets with initial deposits of $0.5 \mu\text{g cm}^{-2}$ of DDT were also exposed out of doors in a Stevenson's screen for 43 days during November and December 1969, when average maximum and minimum temperatures were 7.0° C and 1.3° C respectively. Initial counts of about 200 c.p.s. decreased to 80 c.p.s. at the end of the period. The mean rates of loss in the two experiments were $2 \times 10^{-3} \mu\text{g cm}^{-2} \text{ h}^{-1}$ and $3 \times 10^{-4} \mu\text{g cm}^{-2} \text{ h}^{-1}$ in the laboratory and Stevenson's screen respectively.

In a third experiment, three 40 cm × 50 cm enamelled metal dishes were each given a deposit of 0.5 $\mu\text{g cm}^{-2}$ of DDT. Two of the dishes were placed in subdued light at about 20° C and exposed to a continuous flow of air of about 10 m.p.h. from a fan for 14 days, after which the dishes were washed with 500 ml. of ethanol and, after concentration, aliquots were spotted on planchets for radioassay. Recoveries were 33% and 26% of that obtained from the third control dish, which was washed as soon as the applied solution had dried. The mean rate of loss from the dishes was $1 \times 10^{-3} \mu\text{g cm}^{-2} \text{ h}^{-1}$.

A further experiment was designed to recover and identify the volatilized radioactivity. Eight planchets were given an initial deposit of 0.5 $\mu\text{g cm}^{-2}$ of DDT and covered by clean inverted planchets, which were kept apart by cardboard washers. The inverted untreated planchets acquired radioactivity with a mean count of 20 c.p.s. after 14 days at about 20° C. The radioactive contamination was removed by Soxhlet extraction with ethanol, and after concentration by rotary evaporation aliquots were spotted for thin-layer chromatography on silica-gel covered plates and run in cyclohexane: chloroform (80 : 20). When scanned, one radioactive peak was obtained with an R_F corresponding to that of the original DDT- C^{14} . A similar result was obtained when the plates were run in hexane.

The experimentally determined evaporation rates were in good agreement with the values predicted by the two methods of calculation which, converted to a field scale, suggest losses at the rate of about 2 pounds acre $^{-1}$ yr $^{-1}$ in summer and about 0.3 pounds acre $^{-1}$ yr $^{-1}$ in winter. The interesting implication is that about half the DDT applied to field crops may enter the atmosphere.

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Of Mice and Means

THE use of control and experimental groups of animals for the study of *in vivo* effects of drugs and foreign chemicals is a standard practice. Considerable care is given to several accepted precautions such as the establishment of identical conditions for both groups and the choice of litter mates of the same sex. If a drug is to be given parenterally to the experimental group each corresponding control animal must receive an injection of equal volume and ionic composition given by the same route. Less attention is usually paid to the segregation of the animals during the experiment. A common method is for several animals to be kept in a number of separate cages. The first animal taken from each cage may be used for control purposes and the remainder given different doses of the drug. Alternatively either one cage of animals may be designated the control group and other cages used for a particular drug dose or the control and experimental animals may be distributed randomly throughout the cages. In all these procedures it is implicit that the order in which the animals are used should not affect the biological system being measured. We have obtained some results which suggest that this assumption is not justified.

It was noticed in the course of some work concerned with the

incorporation of radioactivity from labelled amino-acids into the protein of mouse liver that there was a trend in the results obtained with the control animals. This trend seemed to depend not only on the order in which individual animals were taken from a particular cage but also on the order in which the cages were used. Further experiments were therefore made, in each of which three cages, each containing four mice, were used. The animals were given an intraperitoneal injection of 0.5 ml. of 1.3% NaCl solution at 4 min intervals and immediately transferred to individual containers. The order in which the animals were taken out from each cage and the order in which the cages were used were carefully noted. After exactly 1 h each animal was killed by cervical fracture, the liver was removed and the incorporation of ^{14}C -histidine into the proteins of the post mitochondrial fraction determined by standard methods¹.

The results (Table 1) show that the incorporation of radio-carbon into the isolated proteins decreased both with the order in which the animals were taken out of the cages and with the order in which the cages were used. There are statistically significant differences between the results obtained with the first and last animal removed from each cage and between those measured in the animals originally housed in the first and third cages. Similar effects were also observed in experiments in which labelled amino-acids were injected into intact mice.

Table 1 Incorporation of ^{14}C -Histidine into the Proteins of the Post Mitochondrial Fraction of Mouse Liver

Order of removal of animal from cage	^{14}C incorpora- ted (c.p.m./mg protein)	Order in which cages were used	^{14}C incorpora- ted (c.p.m./mg protein)
1	344 ± 105 (10)	1	352 ± 72 (14)
2	284 ± 69 (9)	2	277 ± 76 (13)
3	271 ± 71 (10)	3	232 ± 45 (12)
4	261 ± 61 (10)		

The results are given as means ± s.d.s., the number of animals used being shown in parentheses. They have been analysed by the *t*-test and a statistically significant difference ($P < 0.05$) was found between the first and last animals taken from each cage and between the animals in the first and third cages.

One implication of the present work concerns the interpretation of the results of experiments designed to reveal the possible effects of an agent on protein biosynthesis in the mouse. If either the first animal from each cage or the first cage were chosen as controls, then an inactive substance may have yielded spuriously positive results. This error would have been compounded if the first animals were control and the subsequent mice received increasing doses of the test material. The opposite could also occur in that the effect of an inhibitor could be masked if the last animal from each cage or the last cage were the controls. Although our experiments have been restricted to the determination of one aspect of protein synthesis in mice the possibility that similar effects occur with other biological parameters in laboratory animals normally maintained in small groups in cages must be considered. Any experimental design should take into account the possibility that the order in which the animals are separated into control and test groups may influence the results independently of the agent or procedure being investigated.

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BOOK REVIEWS

Cultural Excavations

The Order of Things: an Archaeology of the Human Sciences. By Michel Foucault. Translated from the French. Pp. xxiv+387. (Tavistock: London, November 1970.) 75s.

EVERY intelligent man must wish on many occasions to have been born French. The elegance and lucidity of the language match the iridescence of the thought. *The Order of Things* is translated (anonymously) to give this atmosphere, though—so far as I can tell—the grasp of technical terminology, certainly in economics, is not too hot. What has Foucault to say? At first sight, like Lévy-Strauss or Teilhard, it is one of those confections that dissolve within the steady breath of linguistic analysis, and the terminological imprecision is no help in that respect. Yet, behind it all, there is something that deserves serious concern, that is just not to be dismissed as another Bergsonian verbal fog or a sort of pointillisme of the intellect.

The book begins with an already classic verbal delineation of Velasquez's *Las Meninas* in the Prado, with its complex reflected images. The point he makes is that in the classical age—Renaissance to Romanticism—the controlling motive was one of order and balance, and a static view of human society and its ideas prevailed. For, according to Foucault, whereas the exact sciences are what they say they are, the social sciences are placed in that part of the atmosphere that lies between *terra firma* and the way that men view the world—their “facts” can only be perceived through a culturally coloured microscope. As the culture changes, therefore, so does the way we perceive and study it—hence the phrase “cultural archaeology” with its suggestion that as we dig back we come across artefacts of other ways of seeing the world.

Accordingly, the argument runs, man as a subject of study and man as an enduring historical thing is a recent post-classical—post-Mannerist one almost says—Romantic and Modern preoccupation. Those disciplines which we know as the social sciences—economics and psychology—represent a manifestation of this way in which the social world perceives itself, rather than a perception of the social world as it in fact is (as physics, say, is of the physical world). These disciplines, this way of looking at the question, are, according to this view, transient and indeed inconsistent; man as an object of his own study may be

passing. Why is this? But first let it be said that the radical distinction drawn between the physical and the social sciences is an attractive one, and it rings true, though one suspects that the place of mathematics in both has not been as centrally in Foucault's thought as it would be to an English-speaking writer on these fascinating topics. If the social sciences, like styles of art, are evanescent, both being ways of looking at the social world, both indeed being the social world, then the reason lies in the increasing formalization of the structure of knowledge—in, say, Russell and Frege—and the awareness of what in shorthand would be called the unconscious, as in Freud and Lévy-Strauss. Language itself, which is the mode of perceiving the social world, has (according to Foucault) changed its nature. Discourse has ended; a more fragmentary and in parts more formalistic series of languages has superseded it. Objectivity is over.

That, at least, is how I would read what is an exceedingly complex argument that seems to have structuralist connotations, though structuralism is indignantly rejected in the preface. “Western culture has constructed, under the name of man, a being who, by one and the same interplay of reasons, must be a positive domain of knowledge and cannot be an object of science” (pp. 366–367). In the struggle, the cry, the madness (the book appears in a series edited by R. D. Laing) a “return” to language is occurring (p. 384), which could be perceived as a return to discourse, though, as that cannot be, Foucault argues, it is man himself as the centre of preoccupation who is disappearing, and new forms of thought will appear to replace the social sciences and all that is implicit in their view of the social world. The argument is exciting and, up to a point, appealing, especially when it deals with the “thinness” of the philosophical content of the social sciences; yet, as so often in French thought, it is clear to an Anglo-Saxon that the elaborate structure of metaphor, embracing abstract nouns, is more rhetorical than analytical. JOHN VAIZEY

Teaching for Teachers

Teaching and Learning in Higher Education. By Ruth Beard. Pp. 222. (Penguin: Harmondsworth, Middlesex, October 1970.) 8s.

TEACHERS in higher education have been waiting for some time for a book on

teaching and learning especially written for them. And who better to write it than Dr Ruth Beard, who has been in charge of the London Institute of Education's pioneering University Teaching Methods Research Unit since its inception in 1965? It was therefore with warm anticipation that I began her book but, in the event, I found it dull and disappointing.

Essentially, the book is in four parts. One part deals with the psychology of learning, another with the planning of courses, a third with teaching techniques, and the fourth with assessment.

The psychology of learning is a diffuse and confused field. In one chapter Dr Beard manages to bring in eight varieties of learning, three types of learning theory and various situational and personality variables, and so it seems carping to complain that anything has been left out. But what is missing is any sustained attempt to relate all this to teaching. Bald statements like “lack of interest tends to follow passive listening” do not help very much.

This excursion into the psychology of learning is, however, only a digression from the principal theme, the development of the paradigm: objectives—activities—evaluation. On this view the curriculum is a contrivance for changing student behaviour; its objectives are statements of desired changes; its evaluation, measurements of changes which have taken place. The author rightly stresses the importance of clear intentions, but the examples which she gives of the application of the paradigm—and there are twelve pages of them—are disappointing. Too often the spelling out of objectives seems to have led to little more than: activity, “all parts of the course”; evaluation, “all forms of assessment”. A detailed and critical appraisal of these attempts would have been very instructive.

From objectives, Dr Beard turns to activities. A large part of the book is given over to advice on lectures, practicals and other teaching methods. Most of this is sound, if sometimes banal (for example, “. . . consult the plant manager or the field-centre warden as to the most suitable time for the visit”). Detailed discussion of problems, such as the generality and specificity of teaching methods and their relationship to objectives, is avoided.

In considering evaluation, emphasis is laid on the important distinction between assessment to help students, “feedback” (which they want more of), and assessment for judgmental purposes (which

they are afraid of). Saving her best contribution until last, Dr Beard has some sensible things to say about the assessment of staff by students.

Somebody once contrasted books which are commissioned by publishers and books which the author feels impelled to write. A weakness of the former tends to be that while they dutifully cover the ground they lack inspiration. Dr Beard's opening words are: "This book was written because I was invited to write it." Perhaps we ought not be surprised then that she has produced for us a comprehensive, but, on the whole, unilluminating account of teaching and learning in higher education.

ALAN SMITHERS

Two Hundred Years of Medicine

Oxford Medicine. (Essays on the evolution of the Oxford Clinical School to commemorate the Bicentenary of the Radcliffe Infirmary, 1770-1970.) Edited by Kenneth Dewhurst. Pp. viii+212. (Sandford: Oxford, 1970.) 50s.

THE Radcliffe Infirmary was opened in the October of 1770; the publication of this book by the distinguished Oxford historian of medicine, Kenneth Dewhurst, is one of the events to commemorate the occasion. By way of an addition to the official history of the infirmary by A. H. T. Robb-Smith, Dr Dewhurst has had the commendable idea of collecting together previously published papers which deal with the evolution of clinical medicine in Oxford. Many have appeared in journals with but a limited circulation and, as Dr Dewhurst rightly states, they merit the attention of a wider audience.

The essays follow a historical pattern which begins with a broad survey of Oxford medicine and goes on to treat in some detail the seventeenth century precursors and the eighteenth century founders of the clinical school. There is then a gap until an account of Acland, who was Regius Professor of Medicine from 1858 to 1895. The Oslerian period, 1905 to 1919, receives close attention and is perhaps the most delightful part of the book, for the contributors have concentrated their approach on personal reminiscences rather than treading the usual and well worn Oslerian pathways. Telling the story of Oxford medicine in the way that has been chosen makes it unavoidably uneven and episodic, but the book next includes a portrait of Sir Archibald Garrod (Regius Professor, 1920 to 1928), a greatly underrated physician whose accomplishments as assessed today would no doubt be worthy of a Nobel Prize. Four articles follow which are the most controversial of the whole series, dealing

as they do with the Nuffield benefactions. This financial infusion stirred the university and the Radcliffe physicians to interminable controversy, highlighted by Lord Nuffield's offer of several million pounds to change the name of the hospital to the Nuffield-Radcliffe Infirmary. The final historical sequence in the development of Oxford medicine is by no means the least, for it includes the story of the first use of penicillin in man, a sketch of Hugh Cairns, the principal architect and catalyst of the present clinical school, and Professor L. J. Witts's thoughtful essay on "Success in Medicine" which should be read by every young medical man.

Dr Dewhurst has had, of course, to exercise selection and could not present a complete coverage of the history of Oxford medicine. He has done so judiciously, however, and has included papers based on the authors' experience of "a novel or idiosyncratic view of persons or events". It is this approach that has made the book so attractive. Not only will it be of great interest to those who have participated themselves in Oxford medicine, scientific or clinical, but it should reach a much wider audience. It should be a source of inspiration, fascination and information to many people both medical and non-medical, and future historians will be grateful for a convenient collection of such useful material.

EDWIN CLARKE

Microbial Approach to Heredity

The Organization of Heredity. By Kenneth R. Lewis and Bernard John. Pp. ix+241. (Arnold: London, September 1970.) 70s boards; 35s paper.

THE authors describe this book as a molecular and analytical approach to heredity using knowledge derived largely from microbial genetics. It presents a very secure and broadly based foundation in the topics discussed; a number of these would hardly need further elaboration in a degree course.

The book falls into three parts. Part one, "The Chemical Organization of the Genotype", has chapters on the material basis of heredity, nucleic acids, and on the chemical basis of mutation. The second part, "The Genetic Organization of the Genotype", occupies nearly half the book and is the core of the work. Different genetic transmission systems are described. Then follow chapters on mapping in different systems and on the mechanism of recombination. The third part contains a discussion of the expression of genetic information (protein structure and synthesis, enzymes, and the genetic code) and a chapter on epigenetic interactions including complementation, con-

trolling elements and nuclear cytoplasmic interactions.

The book is deceptively small, for the text is very concise indeed; hopefully not too much so for students. Seventeen pages describe in detail non-reciprocal events in *Sordaria*, *Ascobolus*, *Neurospora* and *Saccharomyces* tetrads, and relate these to Holliday's model. Essential diagnostic features and conclusions are often tabulated. There are numerous clear diagrams and tables. Reference is made to 100 original papers and reviews and, in spite of the emphasis on microbial systems, half of the ninety species quoted are neither bacteria nor virus.

The topics are well integrated and cross-referenced. The authors have been successful in that their approach has transcended the biological peculiarities of the various systems used as examples. Without such integration some students store their genetic knowledge in units isolated by system and species. Students could progress directly to recent papers in microbial genetics, or to advanced texts on developmental genetics or population genetics. Several topics more peripheral to the principal discourse are introduced (for example, cytoplasmic heredity, puffing in polytene chromosomes, and paramutation) and these suffer from brevity. In general, though, this book carries very little fat and could form an excellent backbone for a second or third year course in genetics, especially as there is a paperback edition available.

J. R. S. WHITTLE

Views of Deafness

Sensorineural Hearing Loss. Edited by G. E. W. Wolstenholme and Julie Knight. (A Ciba Foundation Symposium.) Pp. x+358. (Churchill: London, 1970.) 80s.

SENSORINEURAL hearing loss is the name given to deafness resulting either from some disorder of the inner ear or, more rarely, from damage caused by a lesion within the ear. More than half the deaf population are so afflicted and, because the disorder affects the neural structures themselves, little hope at present exists of any form of cure.

This disheartening state of affairs led Mr Jack Ashley, MP, who suffers from this form of deafness himself, to persuade Ciba to hold a symposium on the subject in December 1969, the aim being to review present knowledge and to consider the ways in which future research might more effectively be directed. The distinguishing feature of this symposium was its multidisciplinary approach; the participants included physiologists, otologists, pathologists, audiologists, psychologists, geneticists and physicists, each of whom had made some special contribution to the study of sensorineural

hearing loss within his own particular sphere of interest.

The result is a unique publication which looks at the subject from several angles covering such topics as deafness of genetic origin, iatrogenic deafness, occupational hearing loss and the effect of noise on the cochlear structures, the audiological investigation of sensorineural hearing loss, the labyrinthine fluids and their importance in the pathogenesis of sensorineural deafness and electrophysiological studies of the cochlea. All the individual contributions are of a high standard, but it is the discussion following each which sets this book apart. It is the aim of the Ciba Foundation to create an environment which encourages the uninhibited interchange of ideas, and the participants of this symposium have obviously reacted with enthusiasm. No ambiguity has been left unchallenged, nor any fruitful avenue of discussion left unexplored.

At the end, Ashley comments, "If we are to regard the tackling of sensorineural hearing loss as the Everest of deafness, it is abundantly clear at the moment that we are only struggling in the foothills." Although it may be true that the perceptive deaf seeking relief from their affliction will find little comfort in this book, other readers will gain considerable encouragement from the discussions bearing on the equally important topic of deafness prevention. In focusing such a diversity of interests on the problem of sensorineural hearing loss, this book sets a precedent which it would be profitable for others to follow. J. D. HOOD

Inside the Rumen

Physiology of Digestion and Metabolism in the Ruminant. Edited by A. T. Phillipson. (Proceedings of the Third International Symposium, Cambridge, England, August 1969.) Pp. x+636. (Oriel: Newcastle upon Tyne, October 1970.) 120s.

THE particular characteristics of the rumen, its microbial population and the host animal have led to the development of a unique area of study. A variety of distinct academic disciplines have contributed to this field of endeavour; biochemistry, physiology, microbiology, nutrition, anatomy and others. The focus of attention on the subject stems to a large extent from the work of the late Sir Joseph Barcroft and his colleagues at the Agricultural Research Council Unit of Animal Physiology in Cambridge during the period 1940 to 1945. It was therefore particularly appropriate that a symposium dealing with this subject should have been held in Cambridge in 1969 and that the proceedings should be edited by Professor A. T. Phillipson, a prominent member of the original unit and now

something of a father figure to those concerned with the rumen.

The proceedings of the symposium constitute a comprehensive and up to date statement of knowledge in this particular and integrated field of study. It is arranged in the form of some four or five papers given in each of nine sessions. These sessions are well arranged to give a clear account of the subject: they cover physiology (dealing particularly with movements of the alimentary tract), absorption, the young animal, the regulation of intake, microbiology, mineral metabolism, biochemistry (carbohydrate, nitrogen and fat metabolism) and metabolic disorders. International authorities, particularly from Australia and the United States, have contributed excellent summaries of their own particular area of interest, and each session is completed by a contribution from a discussant who places each part in context.

The proceedings have been presented in a most effective form and were clearly well edited. Phillipson was assisted by a group of colleagues authoritative in their particular scientific disciplines as applied to the study of the rumen and the ruminant. The proceedings are of the third international symposium dealing with this subject. They are held at intervals of five years and constitute a valuable series of volumes: a very high standard has been set in this volume which will be difficult to maintain in five years' time. The book constitutes an ideal source of reference for all students of the rumen whether they are undergraduates (agriculture or veterinary) or research workers. The subject, which has brought together so many individual scientific disciplines within an integrated whole, is fully described: its importance is readily apparent when it is recognized that ruminants yield 70 per cent of the world's meat protein and almost all milk and milk products. D. LEWIS

Arguments about Drift

Debate about the Earth: Approach to Geophysics through Analysis of Continental Drift. By H. Takeuchi, S. Uyeda and H. Kanamori. Revised edition. Pp. 281. (Freeman, Cooper: San Francisco, 1970.)

THIS book is a very effective introduction to geophysics, using the controversy over continental drift as a theme. This "debate about the Earth" is still continuing today, although Wegener published his theory of drift as long ago as 1912, and the heat has by no means died down. The study of continental drift embraces all branches of geophysics, and the concept lends itself well to popular account. Just so with this book. The authors, all eminent scientists, have

given a clear and fascinating presentation of the historical development of the hypotheses of drift, from early times to the now familiar ideas of plate tectonics.

There are nine chapters; the first two introduce the reader to Wegener and his work, and initiate the "debate", centering the argument on the problem of a feasible mechanism for drift. Chapters three and four discuss the Earth's magnetic field and the principles of rock magnetism. One of the clearest accounts that I have seen of the "dynamo theory" for the origin of the Earth's magnetic field is given in chapter three. It is these two, and the subsequent chapter, which set the tone of the book. It is a personalized account, with the excitement and doubts of the scientists involved left in. Chapter five deals with the palaeomagnetic evidence for drift, and exemplifies the healthy rivalry between different research groups—in this case Professor S. K. Runcorn's at Newcastle and that of Professor P. M. S. Blackett at the University of London. Chapter six discusses the temperature distribution within the Earth and also some arguments concerning the Earth's origin. Possible mechanisms for drift must be compatible with estimates of available energy, and this automatically leads on to a study of the birth of planets. This chapter shows the important link between cosmology and the earth sciences. Chapters seven and eight are about the evidence from the ocean floors; descriptions are given in chapter seven of the early bathymetric, magnetic and heat-flow surveys over ocean ridges, and some remarks about the mantle convection theory. Chapter eight—the new chapter in this revised edition—is remarkable for its lucid account of transform faulting and seafloor spreading. Chapter nine is by way of summing up.

In all, I recommend this book to those with a non-specialist interest in geophysics although the specialist, too, would benefit from its scientific humour. It is neatly produced—the paperback edition using a bathymetric picture of the Atlantic ocean floor, by Heezen and Tharp, as a front cover design. The Japanese edition which, incidentally, grew from a television programme given by one of the authors, has been ably translated into English, with very few errors, by Mrs Keiko Kanamori.

BRIAN W. DARRACOTT

Modelling Inorganic Molecules

Models in Structural Inorganic Chemistry. By A. F. Wells. Pp. xi+186. (Clarendon: Oxford; Oxford University: London, October 1970.) 55s boards; 28s paper.

THIS unusual book by Professor Wells is quite unlike any conventional account of

structural chemistry. It is, as far as I know, the first text to give a detailed account of methods of constructing different types of molecular models and their application to the understanding of various chemical structures. After a brief introduction to the basis of chemical topology, the second part of the book is cast in the form of problems and constructions with the different types of models, and the third part essentially provides help with, and answers to, these exercises. The first chapter of part two is an account of the construction of the regular and other polyhedra using multiple connectors and rods. The second chapter deals with the use of drilled balls and spokes to build up two and three dimensional nets representing chemical structures. The third treats the packing together of spheres in contact, covering both the conventional closepacking situations and the packing together of sets of spheres of different sizes. I found this chapter particularly helpful and informative; most of the material in it could well be incorporated into an experimental course for first or second year chemists. Chapter four gives an account, through problems, of structures built up from tetrahedra sharing one or more verticals, and chapter five deals in the same way with octahedra. Chapter six contains studies on miscellaneous structures, built largely from octahedra and tetrahedra together.

It is difficult to assess all the model-building exercises without the accompanying model-building kit, which, at £25, seems very costly—chiefly, perhaps, because of the expensive models in 'Perspex' of tetrahedra and octahedra. At present the book must be regarded as an adjunct of the expensive model-building set, which is a pity. The inclusion of a chapter for students on how to build, and where to buy, inexpensive components from which to build models would greatly increase the appeal of this volume.

T. C. WADDINGTON

Raman Spectra Revitalized

Laser Raman Spectroscopy. By T. R. Gilson and P. J. Hendra. Pp. xiv + 266. (Wiley (Interscience): London and New York, July 1970.) 90s.

A FEW years ago, laser physics was an esoteric branch of physical science, frequently overcredited by its initiates with the facility to provide solutions for all manner of experimental problems. The days when every physical laboratory had to have its expensive and temperamental laser system for prestige purposes have now passed; inexpensive and docile lasers are used instead for a variety of "workhorse" jobs. This drift out of the spotlight and into the corps-de-ballet is reflected in the appearance of a number

of books dealing in detail with the application of laser systems to specific physical techniques.

The Raman effect, the inelastic scattering of light by matter, was discovered in the 1930s and was used in the elucidation of atomic and molecular structure. Experimentally, however, high intensity monochromatic light sources were needed which were at that time very difficult to produce. This limitation meant that Raman spectroscopy fell into neglect in the 1940s. The advent of the laser provided an ideal high intensity source and set the stage for a renaissance of the technique.

Gilson and Hendra's book is, to some extent, an avowed academic publicity campaign for this renaissance. Written in a breezy style, the book introduces the reader briefly to Raman scattering and to lasers, describes current commercial systems, then gives a detailed description of the analysis of Raman spectra for various states of matter. The success of the book depends on two criteria. First, the practical details must not date too rapidly. Second, the techniques which laser Raman spectroscopy is likely to replace must be reaching the limit of development. The sections dealing with laser systems are unlikely to date quickly, especially as the authors have considered possible future developments. The experimental techniques described are varied and it seems unlikely that, barring a conceptual breakthrough, their range will be superseded in the near future.

The answer to the second question is still open, but Gilson and Hendra have written a readable and thorough introduction to laser Raman spectroscopy which can be recommended to all lay physicists and chemists interested in this technique.

D. G. C. JONES

Mechanics of Memory

Biology of Memory. Edited by Karl H. Pribram and Donald E. Broadbent. Pp. x + 323. (Academic: New York and London, September 1970.) 107s.

NOBODY could regard memory as an under-reviewed topic in the past few years. Indeed, in spite of the fact that it has become a bandwagon research area, since 1960 there have probably been more review volumes and symposia published than really original papers. But with the neurobiologist Pribram and the psychologist Broadbent editing this book, I felt entitled to expect something worthwhile. The first disappointment is to discover that a large proportion of the papers (we are not told which) were in fact given at the Moscow Psychology Congress as long ago as 1966, and most of the rest, apparently, at a meeting in Washington in 1967. In spite of the delay, they have not been brought up to date; several contain no references beyond 1965

and use words such as "recent" in describing work done in 1963 and 1964. Whether editors or publishers are to blame, there is no excuse for foisting off this collection as an up to date set of reviews. The second disappointment is to discover that a number of the papers are in fact little more than abstracts a couple of pages in length, which cannot do justice to the complexity of the issues or to the contributions of individual workers. If one packs thirty-three authors into such a small number of pages, brevity perhaps becomes inevitable, but the disproportion between Melton's three page review of the vexed issue of the relationship between short- and long-term memory, and thirty pages of inconsequentially detailed description of McConnell's attempts to transfer responses between animals by injections of RNA (a theme reviewed much more comprehensively recently in a volume edited by Byrne), is striking and invidious.

This having been said, there are certainly good things in the book. It is still so difficult to get access in English to reviews of Russian work that the substantial contribution to this book by Soviet scientists is welcome. The studies of Sokolov on habituation in a molluscan giant neurone are of general importance, and the remarkable observation by Vinogradova and her colleagues, that there are in the hippocampus a whole variety of cells which respond in different ways to repetition and novelty of stimulus, including, apparently, some cells which can actually "count" trains of stimuli and register changes in their number, is bound to play a major part in future attempts to build models of memory mechanisms. It is also intriguing to learn, if only for students of acronyms, that a computer simulation of a brain memory mechanism devised by Spinelli at Stamford rejoices in the name of OCCAM, not, so far as I can tell, as a tribute to the razor-wielder of philosophical repute, but derived from Omnium-Gatherum Core Content Addressable Memory.

In the preface to this collection, the editors tell us that "as recently as in midcentury, biologists and psychologists alike despaired of coming to grips with the problem of how we organize our experience into the lasting structures that influence subsequent behaviour. The scientific climate has changed radically during the 1960s; a frontal attack on the problem of memory organization has been mounted".

Well, yes; and repulsed, in large measure, with a good number of assault troops injured in the conflict. We now know it is likely to be a long siege rather than a swift assault. It is a pity that this book neither gives us the excitement of the frontal attack of the 1960s, nor lays the strategy for the siege of the 1970s.

STEVEN ROSE

CORRESPONDENCE

Fear of Enlightenment

SIR,—We were rather surprised by the contents of your editorial "Fear of Enlightenment" (*Nature*, 228, 1013; 1970), more especially by its title. It seems to us that it misinterprets and presents a biased view of our article especially for those of your readers who have not read the original in the October 1970 issue of the *Scientific American*, entitled "Intelligence and Race". It is unfortunately all too easy to misinterpret or place the wrong emphasis on a statement taken out of its proper context. Perhaps we were at fault in assuming that a scientifically oriented audience would interpret our comments as they were meant to be, even if briefly stated, rather than as so often happens in everyday life, exaggerate our views to an extent which was clearly neither implied nor intended. We have nowhere in our article argued, as you state, for a "moratorium" on research on such questions as the genetic component of the race IQ difference, or that these questions "should be put into cold storage". Our remarks were clearly not meant as a proposal to bar well founded research in these areas.

As we stated in our article, its aim was to review, mainly for the non-geneticist, the meaning of race and IQ and the approaches to determining the extent to which IQ is inherited, in the hope that this could act as a basis for the objective assessment of the evidence for a genetic component in race and class IQ differences. One of our major conclusions was that, though we do not by any means exclude the possibility that there could be a genetic component in the mean difference in IQ between races, we believe

that there is no evidence against the notion that environmental factors, many of which doubtless remain to be discovered, could explain essentially all the differences in IQ between blacks and white. It is our view that the only conclusive approach applicable to the study of the genetic component of the IQ differences between the races is that of working with black children adopted into white homes and vice versa, and that it is most unlikely that such studies could be done in a reasonably controlled way at the present time especially in the United States.

We tried, within the limits imposed by the audience which we were addressing, to give the scientific basis for our belief that the problem of the genetic contribution to racial differences in IQ is not solved and is not easily soluble by the techniques available today. This point is not even mentioned briefly in your leading article, but is apparently simply dismissed with a vague expression of the opposite belief, namely, that the solution is somehow at hand. The peculiar remark is also added that we should have more confidence "that a proper investigation would produce the result they desire". This seems a strange way of discussing scientific methods and conclusions. Should you not have countered scientific argument with scientific argument? The only example given, namely, the disappearance of the australopithecines, seems a weak one indeed. Moreover, the worry expressed in your editorial that miscegenation may destroy the chance to carry out such investigations cannot be taken too seriously if one considers the relevant facts and figures, which show how far ahead this possibility still lies.

We expect that the problem will be soluble and will be solved well before the time at which complete miscegenation has taken place.

We should, assuredly, be among the first to welcome that break-through in our scientific understanding of the basis of intellectual ability which might lead, in some at present unknown way, to obtaining precise answers to these very difficult questions. As is well known, however, such break-throughs rarely, if ever, come from a massive investment of funds in any particular line of basic scientific research.

Professor Shockley has repeatedly asked for a major expenditure of funds directed specifically at finding the answer to the question of a genetic component to the race IQ difference and other, in our view, similarly at present unanswerable questions, because of their practical importance. He has, in fact, drawn an analogy with the United States man in space programme (see forthcoming correspondence in the *Scientific American*, January, 1971). Apart from the fact that we can see no way in which such information could or should be used in practice, we believe that at the present time such a major appropriation of public funds would be completely misdirected and could achieve nothing positive. When we talk of not "... particularly encouraging the use of public funds ..." we are referring specifically to this kind of directed effort which occurs at many levels through the earmarking of public funds for particular programmes of research in the basic as well as in the applied sciences. We are not afraid of taking the view of "stout hearted liberalism" you commend to us and which we

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believe was properly represented in our article. It is with this view in mind that we believe that, rather than supporting the sort of programme which Professor Shockley seems to be advocating, the kind of effort put by the United States into getting a man on the Moon would be better directed to solving social and economic problems, racial or otherwise, in terms of the *environment*.

Yours faithfully,

WALTER F. BODMER

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LUCA L. CAVALLI-SFORZA

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If the statement in the article in *Scientific American* that "in the present racial climate of the United States, studies on racial differences in IQ, however well intentioned, could easily be misinterpreted as a form of racism and lead to an unnecessary accentuation of racial tensions..." and the conclusion that "we do not see any point in particularly encouraging the use of public funds for their support" were intended merely as comments on proposals such as that of Professor W. Shockley (complained of in *Nature*, 228, 1013; 1970, but not mentioned in the *Scientific American*), Professors Bodmer and Cavalli-Sforza might have helped their readers by saying so explicitly. Editor, *Nature*.

SIR,—In your recent editorial comment (*Nature*, 228, 1013; 1970) on the suggestion by Professors Bodmer and Cavalli-Sforza that there be a moratorium on public research projects dealing with the etiology of observed differences in IQ scores between races, you ignore one of their main points.

It is a fact that there are many illiberal elements in American society who would be delighted to quote or misquote any so-called objective research in support of their viewpoint. I think the present state of knowledge in this field is such that virtually any research carried out is likely ammunition for the illiberal view, however specious their arguments may be.

Consider, for example, my own subjective impression that the educated professional blacks of my former acquaintance were not as intelligent, overall, as their white counterparts. I formulated this viewpoint while a student of psychology in the United States some 13 years ago, and because I was and am of liberal conviction, I concluded that my impression was caused by unconscious prejudice that I had picked up in some way. Consequently, I never voiced my impression, and maintained stoutly that there surely was no innate difference (intellectually) between whites and blacks. On moving to Britain some eight years

ago, I was soon struck by the reverse impression that educated professional blacks in Britain were, if anything, more intelligent than their white counterparts.

I now believe that my contrary impressions could have a kernel of truth. The American black is descended mainly from the former slave population, and it must be remembered that slaves obtained from Africa were often captured and sold in a trade which lasted for many decades. British blacks of my acquaintance seem to be mainly from Africa rather than descendants of slaves. Could not the selection factors involved in the establishment of the American black population have ensured that their gene pool differed from the blacks remaining in Africa? Surely the blacks who were captured for slaves must have tended to be different from those who escaped or, indeed, from some who acted as captors. High intelligence would undoubtedly help escape efforts.

If my observation is true, the best designed studies carried out in America will only support the illiberal contentions. British blacks cannot be used as controls because of cultural and language differences. Can anyone propose a methodology which would resolve all of these difficulties? In the absence of an adequate methodology, can we justify such research when we are aware of the immoral use that is sometimes made of the results?

Yours faithfully,

A. E. HENDRICKSON

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Information Explosion

SIR,—In a review (*Nature*, 228, 966; 1970), Professor F. A. Jenner refers to "The more than exponential increase in scientific information". There has indeed been some increase, but it is far from being exponential, let alone "more than exponential". There has been a great increase in numbers of those involved in most kinds of research, and a phenomenal increase in expenditure, but the amount of information produced, if by information we mean scientific papers describing original work, has been very disappointing. The most noteworthy change in the past 30 years has been the increasing sterility of research workers, and the way in which so much money has been spent with little to show for that expenditure.

Yours faithfully,

K. MELLANBY

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Obituary

Professor G. Hübscher

GEORG HÜBSCHER, foundation professor of biochemistry in the new Medical School of the University of Nottingham, died suddenly after suffering a heart attack on November 3, 1970.

He was born in Berlin and studied medicine both at the Humboldt University and the Phillips University in Marburg an der Lahn. His interest in biochemistry was soon apparent from his work on the preparation and properties of heart muscle cytochrome c in Professor Kiese's laboratory in the Pharmacological Institute at Marburg. The results of this research were accepted in 1953 in fulfilment of the requirements for the MD degree. He completed his medical training at the St Elizabeth Hospital, New Jersey. Georg Hübscher then joined D. E. Green's department at the Institute for Enzyme Research in Madison in 1954 where his interest in enzymology was fostered by studies on the purification and mode of action of uricase. In 1956 he came to England to work in Professor A. C. Frazer's Department of Medical Biochemistry and Pharmacology in Birmingham where in 1964 he became senior lecturer and later reader. He found scope to develop a dynamic teaching programme in intermediary metabolism and enzymology and to gather together a thriving research team to study the biosynthesis of phospholipids and other glycerides in liver and intestinal mucosa. He soon established himself as an authority in these subjects and was awarded the degrees of PhD in 1957 and DSc in 1964. Among his notable contributions to the field of lipid metabolism were the discovery (with B. Clark) of the pathway of resynthesis of triglycerides in the mucosa of the small intestine by direct esterification of monoglycerides and the isolation of phosphatidic acid from ox liver. More recently, his interests were concerned with pathways of phosphatidic acid and glyceride biosynthesis in microsomal and mitochondrial preparations of rat liver and with the short and long term control of the levels of glycolytic enzymes in the mucosa of the small intestine. He was a member of the editorial board of *Gut*. In 1967, he was appointed foundation professor in the new Medical School at Nottingham. He threw himself with zeal into the tasks of establishing a new and active department.

The tragic death of Professor Hübscher at such an early age has meant a great loss to the new Medical School, whose first undergraduates had arrived only a few weeks before. A man of humanity, integrity and energy, he would have had much to offer future generations of medical and science students at Nottingham.

International Geophysical Calendar for 1971

JANUARY

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30	31					

JUNE

S	M	T	W	T	F	S
		1	2	3	4	5
6	7	(8)	(9)	(10)	(11)	(12)
13	14	(15)	(16)	(17)	18	19
20*	21*	22*	23*	24*	25*	26*
27	28	29	30			

JULY

S	M	T	W	T	F	S
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	(20)	(21)	(22)	23	24
25	26	27	28	29	30	31]

AUGUST

S	M	T	W	T	F	S
1]	2]	3	4	5	6	7
8	9	(10)	(11)	(12)	(13)	(14)
15	16	(17)	(18)	(19)	(20)	(21)
22	23	24	25	26	27	28
29	30	31				

SEPTEMBER

S	M	T	W	T	F	S
			1	2	3	4
5	6	7	8	9	10	11
12	13	(14)	(15)	(16)	17	18
19*	20*	21*	22*	23*	24*	25*
26	27	28	29	30		

OCTOBER

S	M	T	W	T	F	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	(19)	(20)	(21)	(22)	23
24	25	26	27	28	29	30
31						

NOVEMBER

S	M	T	W	T	F	S
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	(16)	(17)	(18)	19	20
21	22	23	24	25	26	27
28	29	30				

DECEMBER

S	M	T	W	T	F	S
			1	2	3	4
5]	6]	7]	8	9	10	11
[12]	[13]	[14]	(15)	(16)	17	18
19*	20*	21*	22*	23*	24*	25*
26	27	28	29	30	31	

1972 JANUARY

S	M	T	W	T	F	S
						1
2	[3	[4	5	6	7	8
9	10	11	12	13	14	15
[16]	17	(18)	(19)	(20)	21	22
23	24	25	26	27	28	29
30	31					

(12) Regular World Day (RWD)

(13) Priority Regular World Day (PRWD)

(10) Quarterly World Day (QWD)
also a PRWD and RGD

6 Regular Geophysical Day (RGD)

[25] Day of Solar Eclipse

* Micropulsation Interval Day

[9 10] World Geophysical Interval (WGI)

[3] Day with unusual meteor shower activity,
Northern Hemisphere

[15] Day with unusual meteor shower activity,
Southern Hemisphere

[24 25] Airglow and Aurora Period

Note: International Hydrological Decade Field Year, April 1, 1971 - Sept. 30, 1972.

British Diary

Monday, January 4

Computational Techniques as an Aid in Physical Metallurgy (two-day meeting) Iron and Steel Institute, at the University of Leeds.

Thames Estuary: Restoration and Preservation of its Quality (6.30 p.m.) Dr D. Train, Society of Chemical Industry jointly with the Industrial Water and Effluents Group, in the Scientific Societies Lecture Theatre, 23 Savile Row, London W1.

The Preservation and Waterproofing of Joinery Timber Prior to Painting (7 p.m.) Mr R. R. Hill, Oil and Colour Chemists' Association, at Hull College of Technology.

The Selection and Training of Engineers Abroad (5.30 p.m.) Mr F. H. Woodrow, Institution of Civil Engineers, at Great George Street, London SW1.

Winter Meeting British Society for the History of Science, at Chelsea College, University of London, Manresa Road, London SW3.

Tuesday, January 5

Application of Test Results to the Calculation of Short-Circuit Levels in Large Industrial Systems with Concentrated Induction Motor Loads (6.30 p.m.) Mr C. B. Cooper, Mr D. M. MacLean and Mr K. G. Williams, Institution of Electrical Engineers, at the University of Leeds.

Dynamic Testing (two-day symposium) Society of Environmental Engineers, at Imperial College, London SW7.

Pork from Breeder to Consumer (two-day meeting) Society of Chemical Industry, Agriculture Group, jointly with the Food Group, at the Meat Research Institute, Langford, Somerset.

Systemic Fungicides (two-day symposium) Society of Chemical Industry, at the School of Pharmacy, Brunswick Square, London WC1.

The Geometry of Small Meandering Streams (5.30 p.m.) Mr P. Ackers and Mr F. G. Charlton, Institution of Civil Engineers, at Great George Street, London SW1.

Wednesday, January 6

Chemical Effects at Bearing Surfaces (three-day conference) Institution of Mechanical Engineers and the Royal Institute of Chemistry, at the University College of Swansea, Singleton Park, Swansea.

Colour Matches which include Equality of Scotopic Luminance Dr D. A. Palmer; **Some Aspects of Defective Colour Vision** Dr K. H. Ruddock (3 p.m.) Colour Group (Great Britain) in the Physics Department, Imperial College, Prince Consort Road, London SW7.

Logic Circuits and their Design in the Solid (6.15 p.m.) Mr D. J. Kinniment and Mr A. Bardsley, Institution of Electrical Engineers, at UMIST, Manchester.

Physiological Transducers for Measurement *in Vivo* (symposium) Institution of Electronic and Radio Engineers, Joint IEE/IERE Medical and Biological Electronics Group, at the Institution of Electrical Engineers, Savoy Place, London WC2.

Recent Advances in Microwave Ferrite Devices (6 p.m.) Mr E. Riches, Institution of Electronic and Radio Engineers, Components and Circuits Group, at 9 Bedford Square, London WC1.

Thursday, January 7

Atomic Absorption Spectroscopy (6.30 p.m.) Mr W. R. Nall, North East Metallurgical Society, at the Cleveland Scientific Institution, Corporation Road, Middlesbrough.

Aviation (7.15 p.m.) Women's Engineering Society, at "Hope House", 45 Great Peter Street, London SW1.

Optical Character Recognition and Allied Techniques (colloquium) Institution of

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Electronic and Radio Engineers, Joint IEE/IERE Computer Group, at 9 Bedford Square, London WC1.

Social Policy in the British Steel Corporation (6.30 p.m.) The Rt Hon. Kenneth Robinson, London Metallurgical Society, at the Royal Institution, 21 Albemarle Street, London W1.

Stream Gauging in Upland Catchments (5.30 p.m. informal discussion) Institution of Civil Engineers, Hydrological Group, at Great George Street, London SW1.

Friday, January 8

Earth Currents and Magnetic Variations (2 p.m.) Specialist meeting of the Royal Astronomical Society, at Burlington House, Piccadilly, London W1.

Printing Inks for Web-Offset (6.30 p.m.) Mr D. E. Bisset, Oil and Colour Chemists' Association, at the Manchester Literary and Philosophical Society, 36 George Street, Manchester 1.

The Work of the SRC Panel for the Instrumentation of Large Optical Telescopes (PILOT) (4.30 p.m.) Ordinary meeting of the Royal Astronomical Society, in the Scientific Societies Lecture Theatre, 23 Savile Row, London W1. Chairman: Sir Bernard Lovell, OBE, FRS.

Monday, January 11

A Psychologist Looks at Induction (5.30 p.m.) Dr N. Wetherick, British Society for the Philosophy of Science, in the Joint Staff Common Room, University College London, Gower Street, London WC1.

Design and Maintenance of Basic Oxygen Steel Plant at Port Talbot (7.30 p.m.) Mr H. P. Boyce, Lincolnshire Iron and Steel Institute, jointly with the Iron and Steel Engineers Group and the Lincolnshire Panel of the Institution of Mechanical Engineers, at North Lindsey Technical College, Kingsway, Scunthorpe.

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